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PHYTOPATHOLOGY

VOLUME XIV NUMBER 12

DECEMBER, 1924

PHYSIOLOGICAL SPECIALIZATION OF USTILAGO HORDEI

JAMES A. FARIS[1]

WITH ONE FIGURE IN THE TEXT

In a previous paper (1) I have reported extensive experiments upon the factors influencing the infection of barley by the covered smut fungus (*Ustilago hordei*). In these experiments very high percentages of infection were obtained over a wide range of soil temperature and acidity and at moistures well within those usually existing when barley is planted in the field. Seed of Hannchen barley was germinated in soils of two moistures (40 and 50 per cent) and four pH reactions at six different temperatures from 5° to 30° C. The averages of the two moistures and four pH values gave 6.7 per cent infection at 5° C.; 60.6 per cent at 10°; 61.4 per cent at 15°; 53.9 per cent at 20°; 35.7 per cent at 25°; and 3.7 per cent at 30°. Still higher percentages were secured when the soil temperature was varied.

Considerable data were also given which could be explained only upon the basis that the covered smut fungus, *Ustilago hordei* (Pers.) K. & S., is made up of several biologic forms, a condition unsuspected in any of the cereal smuts until that time. Nepal barley was very susceptible to a collection of smut from a hooded nuda variety but immune to some other collections, while Hannchen and Summit were resistant to the collection which infected Nepal but proved very susceptible to other smut collections. Nepal barley was very susceptible to a collection of smut from a hooded nuda variety but resistant to a collection of smut from California and one from Virginia. Summit barley was susceptible to the collection from California but somewhat resistant to the Virginia and nuda collections, while Texas Winter was susceptible to the Virginia collection but resistant to the California and Hannchen collections. Hannchen was very susceptible to the collection upon Hannchen but was somewhat resistant to the other three.

Since this is a matter of some economic importance as well as of considerable scientific interest, the studies have been continued and enlarged,

[1] Brooklyn Botanic Garden Contributions No. 40.

using both fall and spring varieties of barley and a number of collections of the covered smut. Using the collections of smut which have previously (1) indicated physiological specialization, and pure line seed of the host varieties which seemed differential in separating these forms, numerous experiments were carried out under both greenhouse and field conditions, with results as reported below.

SERIES I—GREENHOUSE EXPERIMENTS TO DETERMINE THE DIFFERENTIAL HOSTS FOR FOUR BIOLOGIC FORMS OF THE COVERED SMUT OF BARLEY

Four varieties of barley were inoculated with the smut fungus from several sources and germinated under controlled soil conditions in order to determine which varieties were susceptible to various possible biologic forms. All seed used was pure line material selected from smut free rows of the previous season and was germinated in a neutral soil with a moisture content of 60% of its water holding capacity, in constant temperature tanks at 20° C. After the seedlings had passed through the infection period they were transplanted to the greenhouse benches and kept under favorable conditions for barley until maturity. The methods used were those previously reported (1) in considerable detail and will not be repeated here.

For the sake of brevity in expression the collections of smut which have shown consistent differences in their ability to infect certain varieties of barley are referred to by numbers. To this extent the results of the experiments are anticipated before the presentation of the data.

The data secured in series I are summarized in table 1.

The collections of smut previously reported as indicating physiological specialization again showed the same difference in their ability to infect the several hosts. Hannchen barley was susceptible to the smut originally collected on Hannchen, (here referred to as Form I) but was very resistant to the other three collections. Nepal barley was suspectible only to the smut from *H. nuda* (Form II) and Texas Winter to the Virginia smut (Form IV). Summit barley is susceptible not only to the smut collected in California (Form III), but also to Form I in a lesser degree. Form III can readily be separated from Form I because Hannchen barley is resistant to the former.

SERIES II—SUSCEPTIBILITY OF THE DIFFERENTIAL HOSTS TO FIVE BIOLOGIC FORMS OF THE COVERED SMUT OF BARLEY

Tisdale (6) has recently reported that removal of the hulls is an effective method of securing satisfactory infections in the covered smut of bar-

TABLE 1.—*Greenhouse experiments to determine the differential hosts for four biologic forms of the covered smut of barley*

Seed No.	Variety	Biologic Form of the Smut											
		Form I from Hannchen			Form II from Nepal			Form III From Summit			Form IV from Texas Winter		
		No. Plants	No. Inf.	Per cent Inf.	No. Plants	No. Inf.	Per cent Inf.	No. Plants	No. Inf.	Per cent Inf.	No. Plants	No. Inf.	Per cent Inf.
66	Nepal	68	0	0	69	24	34.8	70	0	0	71	0	0
101	Hannchen	75	30	40	76	0	0	76	0	0	76	1	1.3
143	Texas Winter	74	0	0	75	0	0	75	0	0	74	13	17.7
181	Summit	74	21	28.4	70	0	0	74	33	44.6	71	0	0

ley. It then became a matter of interest to see whether or not the removal of the hulls would give high infection in a variety inoculated with spores of a biologic form of the smut which does not infect it under normal conditions. For example, would Hannchen barley become infected by Forms II, III and IV if the hulls were removed? In order to determine this point, as well as to secure additional information concerning the susceptibility of these differential hosts to the smut forms, a series of experiments was carried out in the temperature tanks and in the field. A fifth collection of the smut, which had proved interesting in preliminary trials, was included in these experiments. It has proved readily distinguishable from the other known forms of the smut by infection tests, and, hence, is referred to as Form V.

Four varieties of spring barley were germinated in constant temperature tanks at 10, 15 and 20 degrees centigrade, and after the seedlings had passed beyond the stage when infection is possible, they were transplanted to the field for maturing. The hulls were removed from duplicate sets of the hulled varieties (Hannchen and Summit) before inoculation and run simultaneously with the normal seed. Check field plantings were also made of all these varieties after they had been heavily inoculated with spores of each of the smut forms. Here again the hulls were removed from the hulled varieties and a planting made with such seed inoculated with each of the five forms of the fungus.

The previous fall a planting had been made of Texas Winter barley inoculated by each of these fungous forms. The hulls had been removed from one hundred seeds of this variety before they were inoculated with spores of Form IV of the fungus. The Texas Winter barley was not included in the spring experiments because it will not mature satisfactorily in the spring when germinated at temperatures favorable to high infections, as I have previously shown (1).

The results of this series of experiments are given in table 2.

The small numbers of plants matured in the field sowings of seed from which the hulls had been removed should be noted. Tisdale (6) also records a poor stand with "dehulled" seed sown in the field and suggests that this was due to winter killing. A careful study of this difficulty was made with both winter and spring varieties, the details of which are reported in a later paragraph.

The infection of Hannchen barley by Form I, when the seed was germinated in the temperature tanks and transplanted to the field, are very low. Such low infections have been the rule when winter barleys were germinated in the tanks and transplanted to the field in the fall. As indicated by Table 4, the explanation for this seems to be some peculiarity of the

TABLE 2.—*Susceptibility of the differential hosts to five biologic forms of the covered smut of barley*

Seed No.	Variety	Germinated in	Biologic Form of the Smut									
			Form I		Form II		Form III		Form IV		Form V	
			No. Plants	Per cent Inf.	No. Plants	Per cent Inf.	No. Plants	Per cent Inf.	No. Plants	Per cent Inf.	No. Plants	Per cent Inf.
66	Nepal	Field	116	0	78	48.9	134	0	36	0	127	26.8
		Tank 10° C.	58	0	75	20.0	.....	...	57	0	66	40.9
		Tank 15° C.	135	0	122	40.2	60	0	130	0	56	62.5
		Tank 20° C.	68	0	54	75.9	.....	...	52	0	56	73.2
		Total	377	0	329	42.1	194	0	275	0	305	48.2
101	Hannchen	Field	641	40.4	156	0	180	0	43	0	125	6.4
		Tank 10° C.	73	0	72	0	.....	...	75	0	69	0
		Tank 15° C.	146	1.4	134	0	69	0	136	0	73	0
		Tank 20° C.	44	6.8	43	0	.....	...	61	0	44	0
		Total	904	29.2	405	0	249	0	315	0	311	2.6
101	Hannchen (hulls removed)	Field	18	88.9	22	0	14	0	23	8.8	16	56.2
		Tank 10° C.	60	75.0	58	0	.....	...	67	7.5	61	57.4
		Tank 15° C.	92	82.6	134	0	72	0	61	17.2	116	31.9
		Tank 20° C.	20	75.0	39	2.5	.....	...	37	13.5	52	50.0
		Total	190	80.0	253	.04	86	0	188	12.2	245	43.6
181	Summit	Field	110	11.8	126	0	108	21.3	30	0	131	9.9
		Tank 15° C.	56	32..	57	0	62	33.9	58	0	.....	.....
		Total	166	18.9	183	0	170	25.9	88	0	131	9.9

TABLE 2.—(*Continued*)

Seed No.	Variety	Germinated in	Biologic Form of the Smut									
			Form I		Form II		Form III		Form IV		Form V	
			No. Plants	Per cent Inf.	No. Plants	Per cent Inf.	No. Plants	Per cent Inf.	No. Plants	Per cent Inf.	No. Plants	Per cent Inf.
181	Summit (hulls removed)	Field	12	33.3	34	0	22	50.0	9	0	29	51.7
		Tank 15° C.	49	79.6	35	0	45	77.8	51	0	.....	.....
		Total	61	70.5	69	0	67	68.7	60	0	29	51.7
349	Hooded Beardless	Field	91	0	56	23.2	111	0	102	0	538	33.6
		Tank 10° C.	53	0	55	16.4	.....	...	53	0	58	15.7
		Tank 15° C.	66	0	57	19.3	.....	...	62	0	62	37.1
		Tank 20° C.	38	0	46	34.8	.....	...	45	0	36	41.6
		Total	248	0	214	22.9	111	0	262	0	694	33.5
143	Texas Winter	Field	196	0	186	0	176	2.2	76	32.9	110	0
143	Texas Winter (hulls removed)	Field	.....	...	.....	...	.....	...	18	83.3	.....	...
		Total	196	0	186	0	176	2.2	94	42.5	110	0

subsequent growing conditions. While this variety was not infected by Form IV, it usually shows 1 or 2 per cent with that form (See table 1). The data are adequate and there seems to be no reasonable doubt that these smut forms are fundamentally different in their infection capacities.

SERIES III—THE INFLUENCE OF THE DATE OF PLANTING UPON THE INFECTION OF FOUR VARIETIES OF WINTER BARLEY BY FORMS I, II, AND IV OF USTILAGO HORDEI

Field plantings were made at weekly intervals from September 5, to October 25, 1923. Upon each planting date seed of each of four varieties of barley were inoculated with an excess of spores of each of the three forms of the fungus and then planted in 10 foot rows in the experiment field. Great care was taken to prevent mixture of the spores of the biologic forms. The seed grain had been harvested from smut free rows of the 1923 crop, threshed by hand and inoculated. Over 75 per cent of the smut spores of all forms germinated in water and bouillon in 24 hours at room temperature, and practically all of the barley seed was viable in laboratory germination tests.

Hansee Hull-less is a naked, six row, awned, winter barley but is less winter hardy at Brooklyn than the other three varieties. While there was some winter killing in all rows of this variety, the last planting suffered most because the soil temperature remained so low that the plants were but feebly established when the ground was frozen for the winter. Very few plants of the other three varieties, all of which are hulled, six rowed, awned, winter barleys, were lost. In fact, during the three seasons that eighteen varieties of winter barley have been grown in the experiment field of the Brooklyn Botanic Garden, only two varieties, *i.e.*, Nakano wase and Hansee Hull-less, have not been thoroughly winter hardy.

The results of these experiments are recorded in table 3 and summarized in the graph, figure 1.

It is very probable that the soil temperature at the last planting date was so low that it became the limiting factor in the infection by Biologic Form IV, since my previous work demonstrated that a soil temperature of 5° C. practically prevented infection of Hannchen barley by Form I of this fungus. The results of this series of experiments make it quite clear that the four varieties of winter barley used are very resistant to Biologic Forms I and II, and that they are rather susceptible to Form IV. These relations are maintained throughout the varying conditions of soil moisture, temperature, etc., of the entire planting season for winter barley in this locality.

TABLE 3.—*Date of planting experiments. 1923*

Date of Planting	Seed No.	Variety	Biologic Form of Smut								
			Form I			Form II			Form IV		
			No. Plants	No. Inf.	Per cent Inf.	No. Plants	No. Inf.	Per cent Inf.	No. Plants	No. Inf.	Per cent Inf.
September 5	133	Hansee Hull-less	56	0	0	51	0	0	58	13	22.4
	136	Han River	59	2	3.4	64	1	1.6	87	19	21.9
	142	Tennessee Winter	66	0	0	69	0	0	83	13	15.7
	143	Texas Winter	68	0	0	73	0	0	91	20	21.9
September 12	133	Hansee Hull-less	68	1	1.5	70	0	0	78	17	21.8
	136	Han River	70	0	0	76	0	0	88	22	25.0
	142	Tennessee Winter	75	0	0	91	0	0	92	12	13.0
	143	Texas Winter	83	0	0	89	0	0	92	16	17.4
September 19	133	Hansee Hull-less	100	0	0	70	0	0	50	15	30.0
	136	Han River	111	0	0	120	0	0	73	22	30.1
	142	Tennessee Winter	101	0	0	118	0	0	81	12	14.9
	143	Texas Winter	106	0	0	115	0	0	92	17	18.5
September 26	133	Hansee Hull-less	73	0	0	93	1	1.1	138	28	20.3
	136	Han River	141	0	0	118	0	0	152	41	26.9
	142	Tennessee Winter	147	0	0	127	0	0	146	19	13.0
	143	Texas Winter	186	0	0	196	0	0	122	33	27.0

TABLE 3.—(*Continued*)

Date of Planting	Seed No.	Variety	Biologic Form of Smut								
			Form I			Form II			Form IV		
			No. Plants	No. Inf.	Per cent Inf.	No. Plants	No. Inf.	Per cent Inf.	No. Plants	No. Inf.	Per cent Inf.
October 4	133	Hansee Hull-less	45	0	0	48	0	0	38	15	39.5
	136	Han River	100	0	0	105	1	0.9	81	19	23.5
	142	Tennessee Winter	96	0	0	93	0	0	89	9	10.1
	143	Texas Winter	91	0	0	86	0	0	78	12	15.4
October 11	133	Hansee Hull-less	48	0	0	54	0	0	45	14	31.1
	136	Han River	95	0	0	96	0	0	91	32	35.2
	142	Tennessee Winter	90	0	0	80	0	0	108	17	15.7
	143	Texas Winter	93	0	0	92	0	0	92	22	23.9
October 18	133	Hansee Hull-less	30	0	0	36	0	0	17	7	41.2
	136	Han River	75	0	0	78	0	0	74	8	10.8
	142	Tennessee Winter	86	0	0	78	0	0	67	11	16.4
	143	Texas Winter	80	0	0	83	0	0	75	15	20.0
October 25	133	Hansee Hull-less	45	0	0	60	0	0	22	3	13.6
	136	Han River	75	0	0	78	0	0	74	0	0
	142	Tennessee Winter	75	1	1.4	86	0	0	81	0	0
	143	Texas Winter	80	0	0	82	0	0	88	0	0

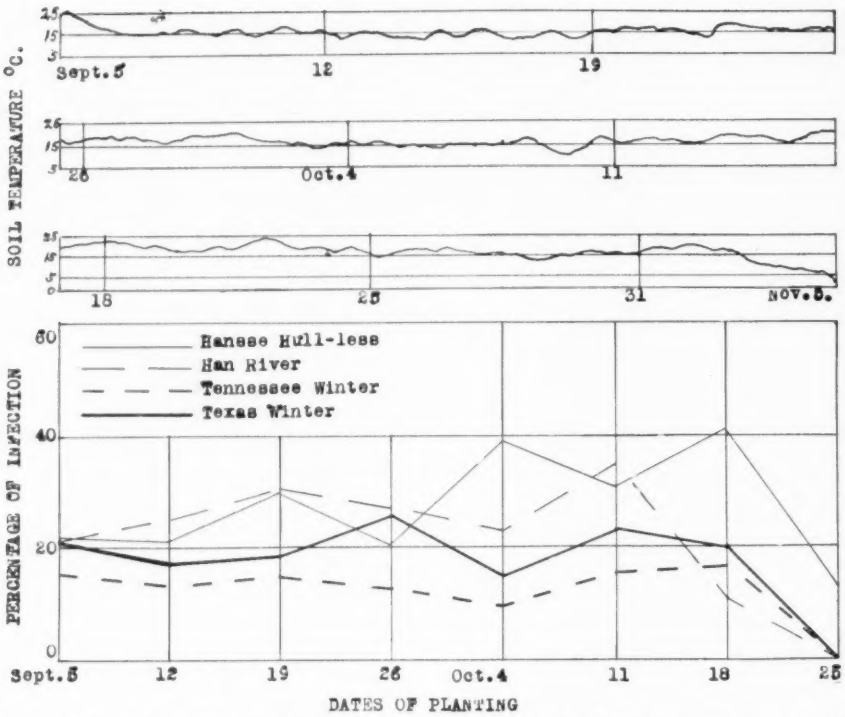

FIG. 1. *Top*—Soil thermograph records from September 5 to November 5, 1923, reduced to one fourth the original size by a pantograph. *Bottom*—Graph showing the percentages of infection of four varieties of winter barley by Form IV of *Ustilago hordei*.

The temperature and moisture records for the season also indicate that these factors greatly influenced the amount of infection by Form IV secured at the various seeding dates. The first planting was made in a dry soil, the second and third in a medium dry soil, the fourth in a soil with a moisture content between 60 and 70 per cent of the moisture holding capacity. The plantings for October 4, 11, and 18 were made in a soil with a moisture content of 40 to 50 per cent, while the last planting was made in a rather wet soil. The soil reaction is between pH 7 and 7.2. The soil temperature fluctuations are shown in the broken graph at the top of figure 1. This graph was made by reducing the weekly soil thermograph records with a pantograph to one fourth of their actual size. All plantings were germinated at temperatures well within the range for high infection (10° C.–25° C.) except the last one made on October 25, which had not emerged before the soil temperature dropped to freezing.

Considering the interaction of these factors and their probable result, these dates of planting experiments are entirely in accord with the results

of the controlled experiments previously reported (1). A certain amount of fluctuation in field plantings is inevitable due to unevenness of the soil, manuring, moisture distribution, etc.

SERIES IV—THE INFLUENCE OF GROWTH CONDITIONS SUBSEQUENT TO THE INFECTION PERIOD UPON THE DEVELOPMENT OF COVERED SMUT

To determine the influence of growth conditions subsequent to the infection period upon the development of this smut, two varieties of winter barley were inoculated with spores of Forms I, II and IV, as in series II, and then germinated in the temperature tank at 20° C. in a neutral soil with a moisture content of 60 per cent of its moisture holding capacity. After these seedlings had passed beyond the stage where infection is no longer possible, they were divided at random and one half planted in the field, the other half in the greenhouse. That is, the conditions during the infection period were the same for both sets of plants and any marked differences in the amount of disease in the final crop must be due to influences of growth after transplanting. The seeds were planted in the tank October 13 and the seedlings were transplanted to the field and greenhouse bench on October 20. The results are given in table 4.

The barley in this series of experiments was planted rather late to avoid the excessively high temperatures of the greenhouse. Because of this late planting some of the seedlings in the field were lost. These results indicate that certain growing conditions after the infection period may have a great deal to do with the amount of covered smut appearing in the final crop. I have reported (1, series III) an experiment in which a number of seedlings were germinated under the same environmental conditions and then divided, one half being matured in a neutral soil and the other half in a strongly acid soil. The plants grown in the acid soil were badly stunted, while the others made vigorous growth, yet there were no marked differences in the percentages of infection. Quite a different set of conditions were under consideration in the present experiment, however, and it would be difficult to say that either the set matured in the greenhouse or that grown in the field was the more vigorous.

It is hard to determine just what factors operated to prevent the development of Form IV of the fungus in the plants matured in the field. It is significant, however, to find that development of the smut may be inhibited after the fungus has penetrated the seedling of a susceptible host, as certainly must have been the case with Form IV of this fungus. The conditions presented in this experiment are very extreme. The plants matured in the greenhouse made uninterrupted growth while those in the field went through the winter "rest period" as do all fall sown grains in

TABLE 4.—*The influence of growth conditions after the infection period upon the development of covered smut of barley*

Seed No.	Variety	Smut Form I				Smut Form II				Smut Form IV			
		No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.
Matured in Greenhouse													
136	Han River	73	0	0	0	71	0	0	0	72	25	3	38.8
143	Texas Winter	74	0	0	0	75	0	0	0	74	9	4	17.5
Matured in Field													
136	Han River	55	0	0	0	59	0	0	0	60	0	0	0
143	Texas Winter	72	0	0	0	59	0	0	0	69	0	0	0

TABLE 5.—*Influence of removal of the hulls upon infection by covered smut of barley*

Where Matured	Seed No.	Variety	Normal Seed				Seed with hulls removed			
			No. Plants	No. Smutted	No. par. Smutted	Per cent Inf.	No. Plants	No. Smutted	No. par. Smutted	Per cent Inf.
Greenhouse	101	Hannchen	75	29	1	40.	68	56	3	86.8
"	143	Texas Winter	74	9	4	17.7	58	53	0	91.3
"	181	Summit	74	31	2	44.6	46	42	1	93.5
Field	127	Omar	82	0	1	1.2	22	5	1	27.2
"	129	Swan Neck	66	2	1	4.5	21	4	3	33.3
"	135	Greece	59	3	2	8.4	33	18	3	63.6
"	136	Han River	86	25	6	36.	14	5	2	50.
"	142	Tenn. Winter	92	10	7	18.4	24	12	1	54.1
"	143	Texas Winter	76	20	5	32.9	18	15	0	83.3
"	145	Wisconsin Winter	72	6	2	11.1	8	1	0	12.5

this climate. The possibility that the conditions of growth of the hosts, after the smut has penetrated the seedling, may influence the percentage of disease in the crop may account for widely divergent results secured in different years, when the soil temperatures and moistures at seeding time would seem to be within ranges to give fairly consistent results. It should be pointed out that the reaction of barley varieties to such environmental changes may not be the same. The growth factors which operated to give very low infections in normal seed of Hannchen barley inoculated with Form I of the fungus as reported in the tank experiments of table 2, did not have a like effect upon Nepal barley inoculated with Form II. Our meager knowledge of the phenomena which may influence the development of the smut after the penetration of the seedling would seem to warrant a series of studies of such problems under controlled light, moisture and temperature throughout the development of the host.

SERIES V—THE INFLUENCE OF REMOVAL OF THE HULLS UPON THE INFECTION AND GROWTH OF BARLEY

Seven varieties of winter barley, all more or less susceptible to Biologic Form IV of the fungus, were planted in 10 foot rows in the experiment field October 3, 1923. The hulls were removed from one hundred seeds of each variety, and they were heavily dusted with spores of Form IV of the fungus. A check row was treated in exactly the same manner, except that the hulls were not removed. These two lots of each variety were planted in adjacent rows. Duplicate seed lots of Hannchen inoculated with Form I, of Summit with Form III, and of Texas Winter with Form IV, were treated in a like manner except that they were germinated in a neutral soil at 60 per cent of its moisture holding capacity and matured in the greenhouse. Only 75 seeds were used in each lot in these greenhouse experiments. The results are recorded in table 5.

The results of this planting confirm those reported by Tisdale (6) upon the infection of winter barley by covered smut in that the percentages of infection were increased by removing the hulls from the seed before inoculation. Tisdale's results were secured in the greenhouse, his field experiments having failed, "due to almost complete winter-killing of plants from dehulled seed." The numbers of plants in my field plantings (Table 5) were also greatly reduced when "dehulled" seed were used. By examination of the rows from time to time it was learned that while practically all the seed from which the hulls had been removed germinated, only a small percentage was able to reach the soil surface. Those which did emerge from the soil were apparently as winter hardy as the adjacent row of normal seed. In many cases such a barley plant is unable to reach the soil sur-

face and the young leaves grow for a time in a twisted, contorted manner, develop a sickly, yellowish-green color, but soon die.

Those plants which reach the surface in a reasonable length of time seem to develop normally. The first leaves are often thicker and in some cases the germination stages up to the development of the third leaf were more advanced in the seed from which the hulls had been removed than in the normal seed. This difficulty was largely overcome in the greenhouse plantings by germinating the seed in the temperature tank and upon transplanting them, straightening out each seedling. This same method was used in series II but, even when this was done, some of the seedlings were lost.

It should be emphasized, however, that in no case was I able to get higher infections under these conditions by removing the hulls from the seed of Hannchen barley (Tables 2 and 5) than I have previously reported (1) for this variety when the hulls were not removed but environmental conditions more favorable for the smut were used. The infections secured with Hannchen barley under the controlled conditions as reported in tables 1, 5 and 6 compare very favorably with those secured at the same soil temperature and acidity in my previous experiments. The removal of the hulls, while effective in securing high infections, creates favorable conditions for the smut which can readily be duplicated by other means, such as those described in my previous paper (1, page 204, tables 3 and 4).

I have no doubt, however, that in farm practice injury to the hull over the seed embryo may play a part in the amount of loss from this barley smut. In fact, I have received field threshed barley with a considerable proportion of the seed as effectively dehulled by close threshing as could be done by hand. Using such grain for seed would not only increase the likelihood of smut loss, but the stand in the field would be much thinner than if uninjured grain were used. In my field plantings of seed with the hulls removed the latter loss was far greater than the smut loss (see table 5).

ENVIRONMENTAL RELATIONS

In a previous report (1) the influences of temperature and acidity at two soil moistures were discussed. The character of the substrata during the period of infection, and wider ranges of soil moistures have been made the basis for further experiments.

SERIES VI—THE INFLUENCE OF THE CHARACTER OF THE SUBSTRATA AND THEIR MOISTURE CONTENT DURING GERMINATION UPON THE INFECTION OF NEPAL AND HANNCHEN BARLEY BY COVERED SMUT

For this series of experiments, two varieties of barley with two forms of the fungus have been used. Nepal, a six-rowed, hooded, naked barley was inoculated with Form II and Hannchen, a two-rowed, awned, hulled barley was inoculated with Form I. These were germinated in the constant temperature tanks at 20° C. In one set of experiments a pure quartz sand, graded to pass through a 20 but not a 30 mesh sieve, was used, and in the other set a good rich potting soil was used. After the period of infection both sets of experiments were transplanted to the greenhouse bench. The results are recorded in table 6.

In both varieties the percentages of infection were much lower when the seeds were germinated in the quartz sand than they were when the neutral potting soil was the medium. The lower moistures of the neutral soil were more favorable for infection than were the higher moistures, a condition noted in my previous report. Lower soil moistures were not used because the seeds would not germinate and higher soil moistures were omitted because of the difficulty of handling soil with a moisture content above 70 per cent of its water holding capacity. The low infections secured in the quartz sand are quite similar to results secured in extensive experiments with covered smut upon both winter and spring varieties of barley when builders sand was used. On the other hand this same builders sand proved quite satisfactory for use with the smuts of oats and sorghum, as reported by Reed and Faris (4, 5).

In view of the low percentages of wheat bunt secured by Heuser (2) in plots treated with nitrogen and complete fertilizers as compared with those secured in plots treated with phosphoric acid, potash and untreated, it would be interesting to carry out a series of experiments in which various nutrients were added to this quartz sand to determine the influence of different fertilizer materials upon infection of barley by *Ustilago hordei*.

Like soil temperature and soil acidity, the soil moisture range for high infection covers all the ordinary moisture conditions for field planting, as it is quite unlikely that barley would be planted in a soil as dry as 20 per cent nor as wet as 70 per cent of its moisture holding capacity.

SERIES VII—THE INFLUENCE OF THE HOT WATER SEED TREATMENT PRIOR TO INOCULATION UPON THE PERCENTAGE OF SMUT IN THE CROP

A quantity of seed of two varieties of barley, Hannchen and S. N. 349, a hooded, naked six-rowed variety, was presoaked in tap water for eight hours and then immersed for 13 minutes in water heated to 52° C. After

TABLE 6.—*The influence of the character of the substrata and their moisture content during germination upon infection of Nepal and Hannchen barley by Ustilago hordei*

Moisture Content of Substrata	Nepal Barley (S. N. 66) inoculated with Smut Form II								Hannchen Barley (S. N. 101) inoculated with Smut Form I							
	Germinated in Pure Quartz				Germinated in Neutral Potting Soil				Germinated in Pure Quartz				Germinated in Neutral Potting Soil			
	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.
10%	35	12	0	34.2	.....	.....	...	.....	43	1	0	2.4	.....	.....	..	.....
20%	41	7	0	17.0	.....	.....	...	.....	47	0	0	.....	25	11	4	60.0
30%	40	11	0	27.5	46	36	0	78.2	50	1	0	2.0	65	26	4	46.1
40%	44	6	0	13.6	69	50	0	72.4	50	0	0	.....	78	25	5	38.4
50%	43	5	0	11.6	71	49	0	69.0	48	1	1	4.1	80	33	2	43.7
60%	43	6	0	13.9	73	36	0	49.3	50	1	0	2.0	75	32	3	46.6
70%	41	1	0	2.4	64	31	0	48.4	50	0	0	.....	79	14	7	26.5
80%	44	2	0	4.5	.....	.....	...	.....	50	0	0	.....	.....	.....	..	.....
90%	43	4	0	9.3	.....	.....	...	.....	50	0	2	4.0	.....	.....	..	.....

thoroughly drying, the seed of Hannchen was inoculated with Form I, and that of S. N. 349 with Form V of the fungus. Lots of untreated seed of each variety were likewise inoculated with the respective biologic forms. A row of treated seed was planted beside a row of untreated seed, and these plantings were repeated at five different places in the experiment field for each variety. All plantings were made the same day, April 5, 1924. The results are summarized in table 7.

The percentages of infection, based upon the totals, are so close that there is no indication that the seed treatment had any appreciable effect upon the amount of smut. A measured amount of seed was used in each row and the smaller number of plants which were matured in the treated rows indicates that there was some seed injury from the treatment.

DISCUSSION AND CONCLUSIONS

That the covered smut of barley is made up of several biologic forms, as suggested in my previous report (1), is here demonstrated beyond reasonable doubt, and the differential hosts for five forms of the fungus are given. I have referred to these biologic forms by a form number, thus adopting the method used by Dr. E. C. Stakman and his co-workers in differentiating the biologic forms in the cereal rusts.

The infection relations of these five forms to their differential hosts may be summarized as follows:

Seed No.	Variety	Form I	Form II	Form III	Form IV	Form V
66	Nepal	R	S	R	R	S
101	Hannchen	S	R	R	R	S
181	Summit	S	R	S	R	S
143	Texas Winter	R	R	R	S	R

R = Resistant S = Susceptible

Form I infects Hannchen and Summit, Form II infects Nepal, Form III infects Summit, Form IV infects Texas Winter and Form V infects Nepal, Hannchen and Summit.

Each of these varieties has been inoculated with each smut form and germinated under a variety of environal conditions. Their resistance to forms, other than those to which they are listed as susceptible, has been maintained, thus demonstrating that the smut forms are biologically different in their capacity to infect.

The results of preliminary experiments, in which more than 100 varieties of barley were inoculated with smut collections from many localities, indicate that there exist still other biologic forms of this smut. More

TABLE 7.—*The influence of hot water treatment upon the percentage of smut in subsequently inoculated seed*

Planting No.	Hooded beardless barley (S. N. 349) inoculated with smut form V								Hannchen barley (S. N. 101) inoculated with smut form I							
	Seed untreated				Seed treated with hot water				Seed untreated				Seed treated with hot water			
	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.	No. Plants	No. Smutted	No. Par. Smutted	Per cent Inf.
1	61	7	5	19.6	33	8	2	30.3	85	18	24	49.4	64	7	19	40.6
2	37	10	9	51.3	26	3	5	30.7	80	7	26	41.2	77	6	18	31.1
3	46	6	6	26.0	28	4	3	25.0	91	11	23	31.3	73	12	18	41.0
4	74	19	7	35.1	50	11	1	24.0	65	6	25	47.6	64	5	26	48.4
5	73	18	2	27.4	44	14	1	34.0	103	13	30	41.7	93	3	27	32.2
Total	291	60	29	30.5	181	40	12	28.7	424	55	128	43.1	371	33	108	38.0

experimental work is necessary before the relation of these probable forms to the differential hosts of the above five forms can be stated, however.

In view of the finding of biologic forms in the covered and loose smuts of oats which has been reported by Reed (3), and the above results with the covered smut of barley, it would seem quite likely that other smut fungi are specialized into biologic forms. Since some host varieties are susceptible to more than one biologic form of a fungus (*i.e.*, Summit barley to Forms I, III and V), the possibility that smutted heads of such a variety collected in the field may contain a mixture of forms should not be overlooked in experimenting with this disease. It becomes very important to have not only authentic seed of the barley varieties, but also to know something of the host relations of the fungous material to be used, before extensive experiments with this disease are undertaken. A diligent search should also be made with other smut species in order to determine whether or not specialized races exist in them.

ENVIRONMENTAL RELATIONS

The date of planting experiments, in which four varieties of winter barley inoculated with Forms I, II and IV of the covered smut fungus and planted at weekly intervals during the fall of 1924, indicate that the soil conditions became more favorable for infection from early September to late October, at which time the cold soil temperatures became the limiting factor in infection with Form IV of the parasite. These barley varieties maintained their resistance to Forms I and II of the fungus at all of the various planting dates.

There are considerable data in tables 2 and 4, which indicate that growth conditions after the infection period may have a very marked effect upon the percentage of smut which finally appears in the crop.

The "dehulling" of the seed, either by hand or by close threshing, may increase not only the likelihood of smut infection but may be a source of even greater loss because of a poor stand of barley.

The character of the soil has proven to be of considerable importance in securing satisfactory infections with covered smut. Quartz and builders sand have been found poor substrata for securing high infections and in this respect this disease has proven quite different from the smuts of oats and sorghum reported by Reed and Faris (4, 5).

The results of the moisture studies reported in table 5 indicate that high infections may be gotten over wide ranges of soil moisture. As pointed out previously (1), the final amount of disease appearing in the crop is the result of the interaction of many factors. The present results serve to emphasize that the factors limiting infection may be environal or

biologic and may exert their influence upon the parasite or the host or the parasite relation between the fungus and the host.

The treatment of the seed grain by hot water before inoculation with the fungous spores, had no appreciable effect upon the percentage of smut secured in the field plantings reported in series VII. It would seem, therefore, that experimental results secured with seed treated by hot water would be comparable to results from smut free seed which had not been treated.

The writer is indebted to Dr. G. M. Reed, Curator, Brooklyn Botanic Garden, for many valuable suggestions during the course of this work; and to Prof. R. A. Harper, who generously permitted the use of some of his private grounds for carrying out the extensive preliminary experiments.

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PATHOLOGIC HISTOLOGY OF APPLE BLOTCH

E. F. Guba

With Plates XXX and XXXI and Five Figures in the Text

The history of the Phyllosticta apple blotch, caused by *Phyllosticta solitaria* E. & E., began with collections of this disease by Underwood (7) from Crawfordsville, Indiana, in 1893, on the leaves of the native American crab apple (*Pyrus coronaria* L.) and by Clinton (1) from southern Illinois in 1902 on the fruit of the commercial apple (*Pyrus Malus* L.). In 1920 Roberts (3) presented a brief account of the pathologic histology of apple blotch cankers. To the knowledge of the writer this appears to be the only published account on this subject. The object of this paper is a further and more extensive consideration of the pathologic histology of this disease. The main results of the investigation, already published in abstract form, will appear in print subsequently.

When this study was undertaken it was hoped to determine the mode of entrance of the fungus into the healthy tissues and the morbid changes in the tissues immediately following infection. Unfortunately, these points have not been solved by this study for reasons which may be briefly enumerated here. Spore production in culture of species of the genus Guignardia, to which, no doubt, *Phyllosticta solitaria* belongs, appears to be a rare occurrence, this being decidedly so of *Phyllosticta solitaria*. In the absence of a supply of spores from pure culture, artificial inoculations of the host with spores from this source were not possible. Efforts were made to inoculate the host with spores from apparently mature fruiting bodies obtained from lesions on the host but the spores from these sources when available and when needed generally failed to germinate. Scott and Rorer (5) (6) report having obtained successful artificial inoculation in the orchard with spores obtained from natural sources, but their results are open to criticism since the inoculated parts were exposed to natural infection and spores from pure culture were not used. Roberts (4), however, established the pathogenicity of the fungus with spores from pure culture. The accounts of his results are presented rather briefly without affording any definite conclusions regarding spore production in culture or regarding infection. No one has yet determined the manner in which infection takes place. The problem of spore production in culture and artificial infection with spores warrants investigation. In view of the writer's failure to obtain artificial infection, certain points of interest in the pathologic histology of the disease could not be determined. This account, there-

fore, is largely based on the gross histology of affected tissues as revealed in microscopic sections.

IN THE TWIGS

The anatomy of the normal bark of one year old apple twigs in transverse section reveals the cuticle, epidermis, cork layers, a compact, rather broad band of collenchyma of four tiers of cells and a loose spongy parenchyma with many intercellular spaces (Plate XXX, fig. B). Strands of sclerenchyma are located deep in the spongy parenchyma, that is, in the pericyclic region, and beyond them are evident the medullary rays. Within the sclerenchyma and between it and the cambium is the phloem. Frequently in the young twigs individual strands of sclerenchyma are united to form an unbroken irregular sheath of sclerenchyma around the phloem and the terminations of the medullary rays.

A transverse section through diseased bark reveals necrosis extending to a point midway between the epidermis and the cambium. The diseased tissues are separated from the healthy, directly beneath the sclerenchyma,

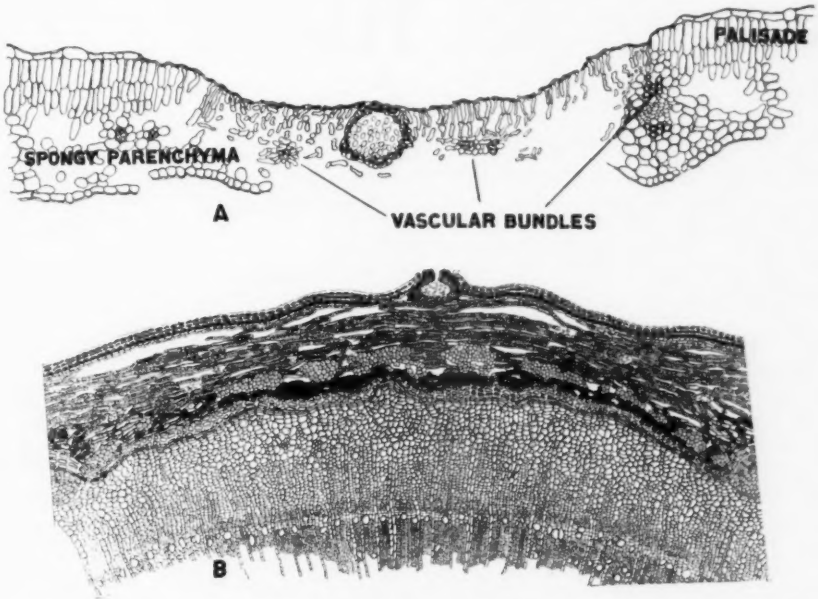

FIG. 1. A. Section through a leaf spot showing the central position of the pycnidium, the dead palisade cells hanging loosely from the collapsed epidermis, the mass of dead parenchyma cells around the vascular bundles, and the compact mass of living cells across the mesophyll at the vascular bundle. B. Section through a canker on a water sprout showing the necrotic region on the outside, the cork cambium, and the dense underlying region of small parenchyma cells (phelloderm). From material collected November 10, 1919.

by a compact cork layer of two to three tiers of cells (Fig. 1, B). Directly outside of this cork cambium is a compact sheath of dark colored cells. The formation of periderm by the cork cambium occurs rapidly. Phellem production is limited to a few layers of cells while, to the inside, phelloderm production is very marked (Plate XXX, fig. A and C). Coincident with the formation of periderm the cambium layer is stimulated in places to rapid formation of parenchyma (Plate XXX, fig. A) instead of normal phloem. The phelloderm is composed of small round cells arranged in compact radial columns (Fig. 1, B) and the medullary rays which extend out through the phelloderm to the cork cambium are distorted and composed of small cells.

The mycelium is intercellular and generally present outside of the abscission layer and its development appears to be most extensive in the collenchyma. The fungus does not come into contact with the phloem, medullary rays and cambium although these tissues appear to be markedly affected indirectly as the result of the necrosis of the outer cortex. Between the lower tiers of cells of the collenchyma and the outermost tiers of cells of the spongy parenchyma, small knots of mycelium are evident, these later developing into pycnidia or pycnosclerotia according as they are formed early or late in the season.

In August and September when the new cankers first became evident and during the course of the following dormant season the diseased parenchyma cells outside of the periderm still appear to be living. The growth of the cankers is to a large extent inhibited during the dormant season. Cracks are formed in the fall especially along the longer margins of the cankers, which in transverse section appear just outside the periderm (Plate XXX, fig. C). The growth of the fungus occurs actively again early in the spring and, as a result, the cells within the invaded region are killed. The presence of the protective layer and the complete isolation of the necrotic tissues, with the resulting exposure of these tissues to desiccation, probably also plays an important part in the death of the cells.

New cankered areas appear along the margins of the old cankers. Whether the fungus is already present in the parenchyma beyond the cork cambium and merely continues its growth when favorable conditions permit or whether it penetrates the periderm and infects the healthy tissues beyond it early in the season has not been determined. In Illinois the growth of the fungus and the formation of new cankered areas appear to be most marked early in the spring and early in the fall. The necrosis of the outer cortex and the formation of cork cambium layers below the selerenchyma continue for some years. The cankered areas become rifted at the margins (Plate XXXI, fig. D) and by being marked with different shades of brown appear

distinct from other portions of the canker. The dead tissues are gradually exfoliated (Plate XXXI, fig. B) thus exposing the cork cells or phellem. The increase in the number of cells between the cork cambium and the cambium as the result of the activity of these meristematic tissues and their growth in size and differentiation continues until the wound is repaired.

It is apparent from the foregoing account and the study of plate XXX, fig. A and figure 1, B that the fungus may be readily removed from the bark with a knife by cutting away all of the discolored tissues. This exposes the fresh healthy phloem between the abscission layer and the cambium and the rapid formation of new phloem by the cambium eventually repairs the wound.

IN THE PEDICELS

A transverse section of the cortex of a normal apple pedicel shows on the outside a thin cuticle, then a narrow epidermis, surmounting a much broader layer of collenchyma of about four to five tiers of cells and next spongy parenchyma of large cells and intercellular spaces. Beyond the parenchyma are regions of sclerenchyma which are united more or less into broad irregular bands around the phloem and the terminating cells of the medullary rays.

The pathologic histology of the pedicel in transverse section generally appears to be similar to that of the twigs (Fig. 2). The necrosis of the pedicel extends midway into the parenchyma. Here, however, the protective layer forms outside of the sclerenchyma sheath rather than inside as was found to be the case in the twigs. The cells of the protective layer are commonly round or broad and elongate with truncate ends exposed to the necrotic area and their walls and contents are brown. There is no indication of the formation of phelloderm or of the presence of a cork cambium. It appears that individual cells of the spongy parenchyma between the protective layer and the sheath of sclerenchyma have divided to account for hyperplasia in this region. Outside of the protective layer the tissues are collapsed and desiccated. This layer prevents drying, affords mechanical protection and hinders the invasion of the underlying parenchyma by the pathogene.

The mycelium is generally distributed throughout the necrotic tissue although most extensively developed in the collenchyma where necrosis occurs at a more rapid rate than in the parenchyma directly beyond. Pycnidia are formed between the upper tiers of cells of the collenchyma or directly below the epidermis.

IN THE FRUIT

The healthy apple when studied in transverse section shows a thick cuticle lying over an epidermis composed of irregular polygonal cells. Below the epidermis lies the hypodermis of about 4 to 5 tiers of cells. The ultimate branchlets of the vascular bundles approach the hypodermis. Beyond, extending to the primary vascular bundles, is a much broader region of spongy parenchyma which with the hypodermis comprise the "cortex" or the outer pericarp. Ten primary vascular bundles lie in a circle about midway between the central cavity and the epidermis and mark the division between the cortex and the "pith" or inner pericarp. The pith is comparatively narrow, composed of spongy parenchyma extending to the endocarp. It is distinguishable from the cortex by the absence of vascular tissue and by the longer, narrower cells.

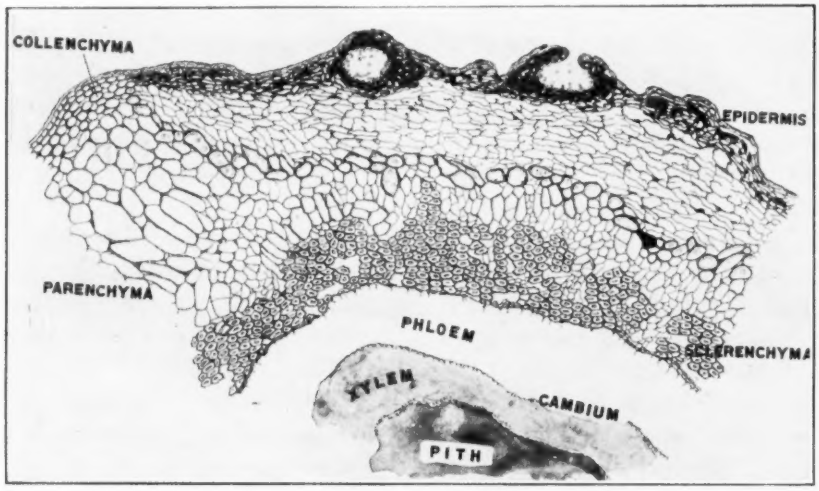

FIG. 2. Section through an affected pedicel. The living and dead areas are separated by a definite protective layer of dark colored cells; below in the parenchyma tissue and outside of the sclerenchyma the tissue is compact and composed of small irregular shaped cells.

The different varieties of apples usually manifest different symptoms of apple blotch which may be classified according to their external appearance as: the pitted; the blistered; and the fringed blotch types. The lesions of the fringed blotch type are radiate and feathery with fringed margins. This type may be found on the Northwestern Greening, Maiden Blush, Yellow Transparent, and Duchess varieties. The pitted type is characterized by sunken areas with definite and distinct borders; it appears to be

common on the Jonathan, Missouri Pippin, Ben Davis, Arkansas Black, and McAfee varieties. This type is excellently illustrated by Lewis (2). The fringed blotch type precedes or sometimes surrounds the pitted areas as they enlarge. The occurrence of deep rifts across the blotches appears to be common to the fringed blotch type. In contrast to the hard and superficial qualities of the lesions of the fringed type, the lesions of the pitted type are deeper and the tissue is soft although also dry. The early stages of apple blotch are usually all of the fringed type; later as the fruit matures some portions become pitted while other portions remain distinctly feathery and spreading. The third type is manifested in the form of blisters with partly cracked margins. This type appears to be common on the May of Meyers and the Benoni.

From a histological study of the affected tissues of apples exhibiting these types of blotch it appears that the reaction of the host cells to the invading fungus is responsible for differences in the symptoms. These different types of apple blotch appear to be quite characteristic of certain varieties. It seems logical to believe that they are the result of differences in the growth of the apple peculiar to the variety rather than of different strains of fungus.

a. *Fringed Type of Apple Blotch*

In the fringed type of apple blotch, necrosis is confined to the epidermal and hypodermal cells. The spongy parenchyma cells directly beyond apparently are not affected. Growth of the apple below the affected portion is stunted while the tissues around the necrotic region continue growth and, as the result, create opposite stresses. The necrosis of the cells of this outer region of the fruit also unduly exposes the tissues to drying. As the result of these abnormal conditions cracks form across the surface of the blotch. The crack at first is broadly wedge-shaped and extends into the hypodermis. In the lower hypodermis the cells are brown and are arranged very compactly. In the spongy parenchyma broken cell walls and radially elongate cavities are evident and ultimately, the crack extends deep into the pith. The cells of the spongy parenchyma adjacent to the rifts become torn and radially elongate, and greatly distorted about the bundles.

b. *Blistered Type of Apple Blotch*

The lesion is quite superficial, confined to the hypodermis and epidermis and limited radially by the formation of distinct periderm directly below the hypodermis (Fig. 3). Directly outside of the periderm is a protective layer of compactly arranged round, dark colored cells. The abscission region inhibits the progress of the fungus radially into the spongy paren-

chyma, although tangentially the fungus grows slowly along the hypodermis causing necrosis of the cells. The raised blistered blotches result from active growth of the cork cambium, giving rise to a broad, compact mass of small cells, the phelloderm, arranged in radial columns. The amount of phellem produced is comparatively insignificant. The epidermis is sometimes broken at the margins of the blotches. As desiccation progresses, the collapsed, discolored cells become more or less separated from the abscission layer.

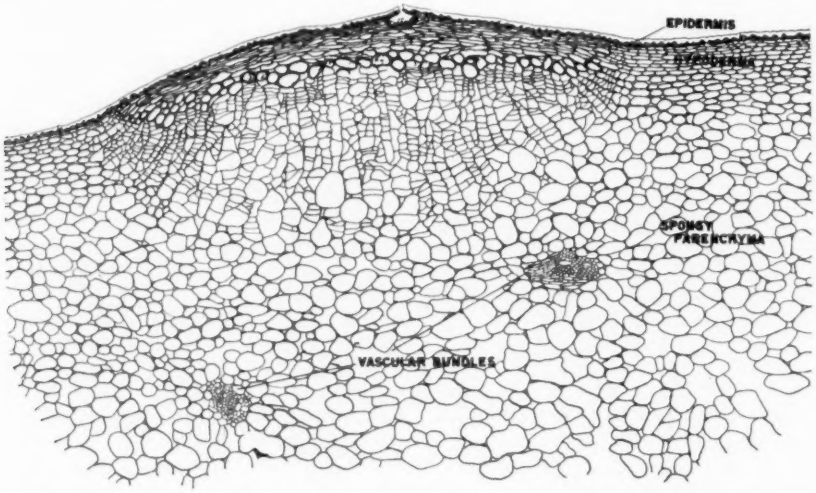

FIG. 3. Section through an apple affected with the blistered type of apple blotch showing the distinct protective layer of large, round, dark colored cells and the periderm layer dividing the living and dead tissues. The dense columnar arrangement of the cells of the phelloderm and their marked multiplication in number is very distinct of this type of apple blotch.

c. *Pitted Type of Apple Blotch*

A marked contrast between the pitted type and the blistered type of apple blotch is indicated by the absence of a distinct abscission layer between the dead and living tissues and in the presence of large cavities in the cortex directly beyond the hypodermis (Fig. 4). Necrosis extends deep into the cortex below the outer vascular bundles. The epidermal and hypodermal cells are collapsed, these tissues together forming a dense, collapsed, region. Below in the spongy parenchyma the cell walls are torn apart, and in the corners of the dead cells masses of starch grains are evident, their formation being the result of disease.

The dead pitted region is sometimes definitely surrounded by normal spongy parenchyma, hypodermal and epidermal cells. Usually the pitted

area is surrounded by an area of blotch of the fringed type, in which case the hypodermis and the epidermis only are affected. The pitted region is dry but soft, due to the large open spaces in the cortex.

The intercellular mycelium is extensively developed directly beyond the epidermis and extends loosely among the cells of the hypodermis and partly into the spongy parenchyma. It is entirely absent deep in the affected cortex. The pycnidia form directly below the epidermis and commonly between the upper tiers of cells of the hypodermis.

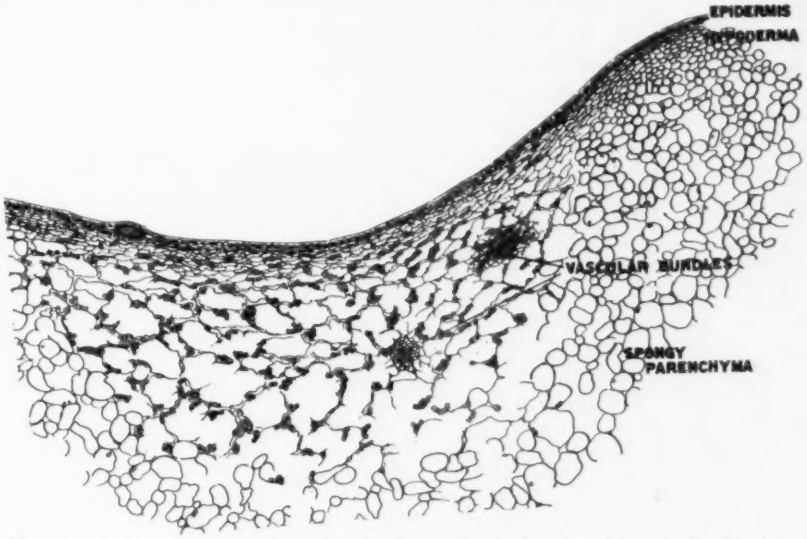

FIG. 4. Section through an apple blotch pit from the Jonathan apple showing the characteristic large, open cavities, the loose broken cell walls in the spongy parenchyma, and starch grains in the cells of the affected region. The living and dead tissues are not separated by a protective layer.

IN THE FOLIAGE

In the Blades

The spots are sunken on the upper surface, and in transverse section the thickness of the leaf is reduced usually to about one-half of the normal (Fig. 1 A). The bundles seem to offer some obstruction to the progress of the fungus. The healthy and dead areas are usually separated by a compact mass of parenchyma cells reaching across the mesophyll, near a vascular bundle. The usually solitary pycnidium occupies a central position on the upper surface of the spot (Plate XXXI, fig. A).

The mycelium is intercellular and ramifies loosely among the palisade tissues and parenchyma cells. It appears to be most extensively developed

below the upper epidermis from which strands extend among the cells of the mesophyll. Infection is followed by the development of a mass of mycelium directly below the epidermis and in the palisade tissue which leads to the formation of the pycnidium. As the pycnidium develops, the dead palisade cells are crushed and pushed aside and the epidermis is raised and ruptured. The upper epidermal and palisade cells collapse as the fungus advances and later the cells of the spongy parenchyma and lower epidermis succumb. The palisade cells become distorted and hang loosely from the dead collapsed epidermis, while those of the spongy parenchyma separate, or are grouped in small masses about the vascular bundles. Occasionally, portions of the lower epidermis and spongy parenchyma fall away, exposing the palisade cells above.

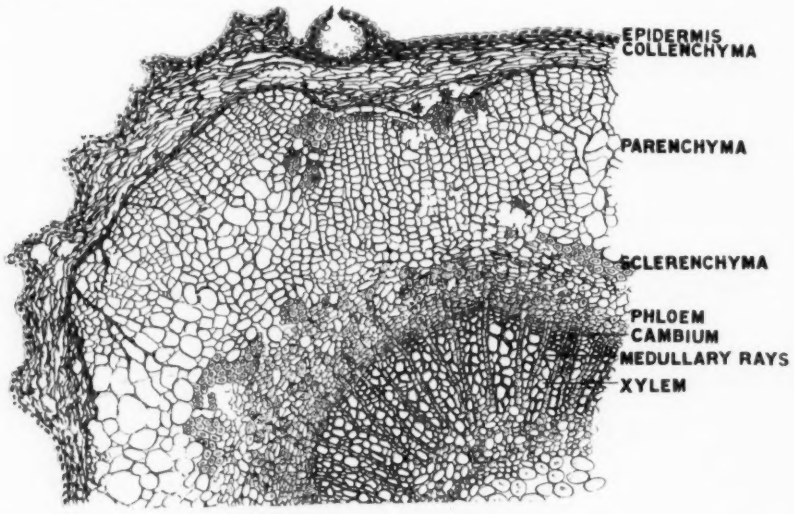

FIG. 5. Section through a petiole showing the effect of the fungus upon the tissues, the dead outer region of the cortex, the abscission layer and the dense columnar arrangement of the parenchyma cells beneath where marked hyperplasia is evident. Strands of sclerenchyma have been pushed out along the abscission layer.

In the Petioles

In the normal apple petiole, the epidermis surmounts a band of collenchyma of two or three compact tiers of cells. Beyond the collenchyma lies a rather broad region of spongy parenchyma the latter bordering on the inside scattered strands of sclerenchyma and phloem. The larger terminating cells of the medullary rays are evident out beyond the phloem.

The diseased region of the petiole is sunken and the surface becomes irregular, dry, and leathery (Fig. 5). In transverse section the epidermal

and collenchyma cells are collapsed. The dead cells are arranged compactly and distorted irregularly. In the spongy parenchyma, the collapse and distortion of the cells is also evident and the cell walls commonly are broken. The necrotic and living tissues of the cortex are definitely separated at a point about midway between the epidermis and the pericycle. The abscission region is sometimes interrupted by strands of sclerenchyma. Here as in the pedicel the protective layer is formed at some distance outside of the sclerenchyma. Underlying the abscission region, in places the cork cambium has given rise to a dense region of parenchyma of small, uniform-sized cells, the phelloderm, arranged in compact, radical layers. In other places the division of the parenchyma cells of the pericycle is evident and appears to account for the presence of strands of sclerenchyma out in the abscission region (Fig. 5). The host is unable to resist the progress of the fungus tangentially and as the result, the epidermis, collenchyma and outer parenchyma may undergo necrosis in this direction until the petiole is girdled. Usually the medullary rays and the vascular elements are influenced by the changes which take place in consequence of which they become somewhat asymmetrically arranged. The same general changes accompany the necrosis of the mid-veins of the leaves (Plate XXXI, fig. C).

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DESCRIPTION OF PLATES

PLATE XXX

FIG. A. Photomicrograph of section through a canker from a water sprout showing the marked response of the cambium to the presence of the fungus. The dense protective layer is apparent directly below the strands of sclerenchyma. From material collected December, 1920.

FIG. B. Section through a water sprout showing the arrangement and construction of the normal tissues. From material collected November 20, 1919.

FIG. C. Photomicrograph of section through a canker at the margin showing the wedge-shaped crack directly outside of the periderm. The cork cambium and phelloderm are very much in evidence. From material collected December, 1920.

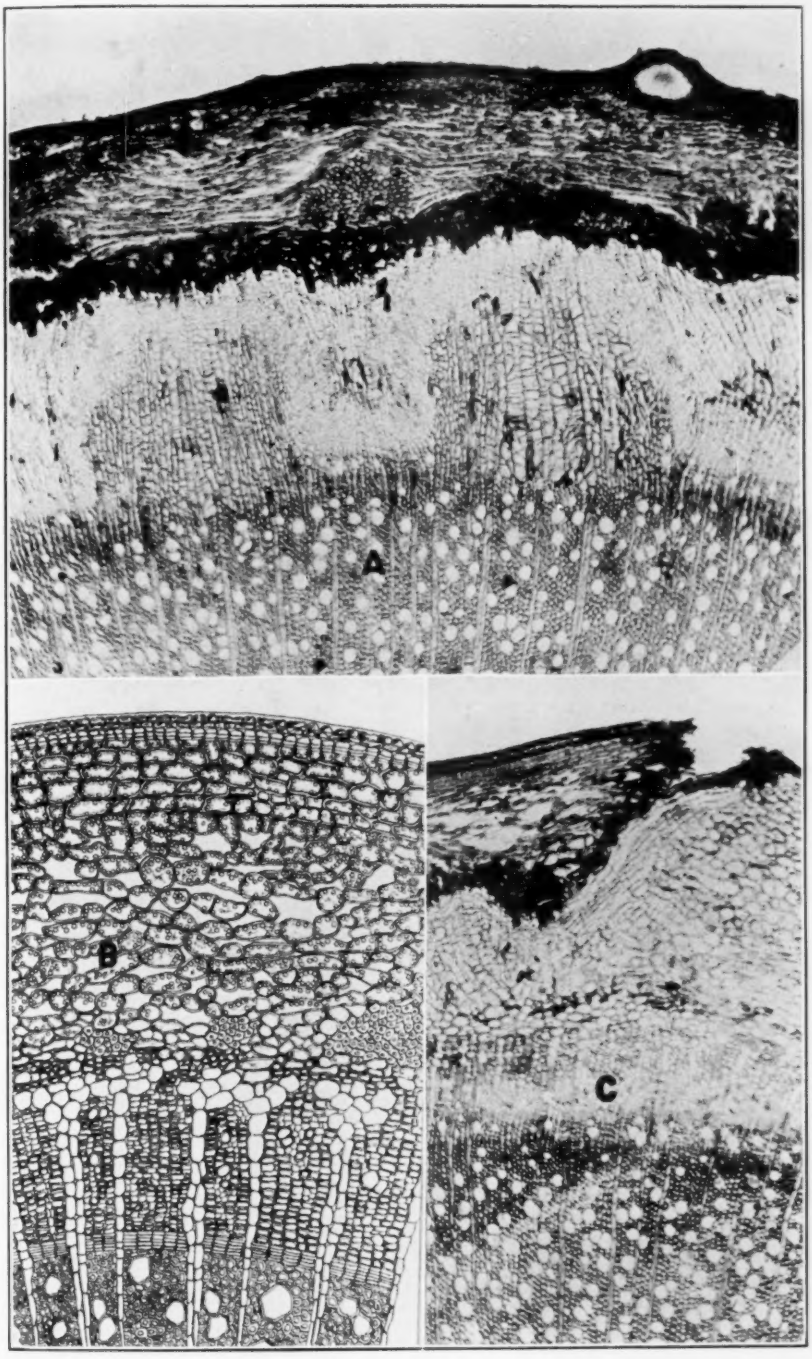
PLATE XXXI

FIG. A. Enlarged view of a portion of an apple leaf affected with *P. solitaria* showing the central position of the usually solitary pycnidium on pale spots of definite sizes and shapes.

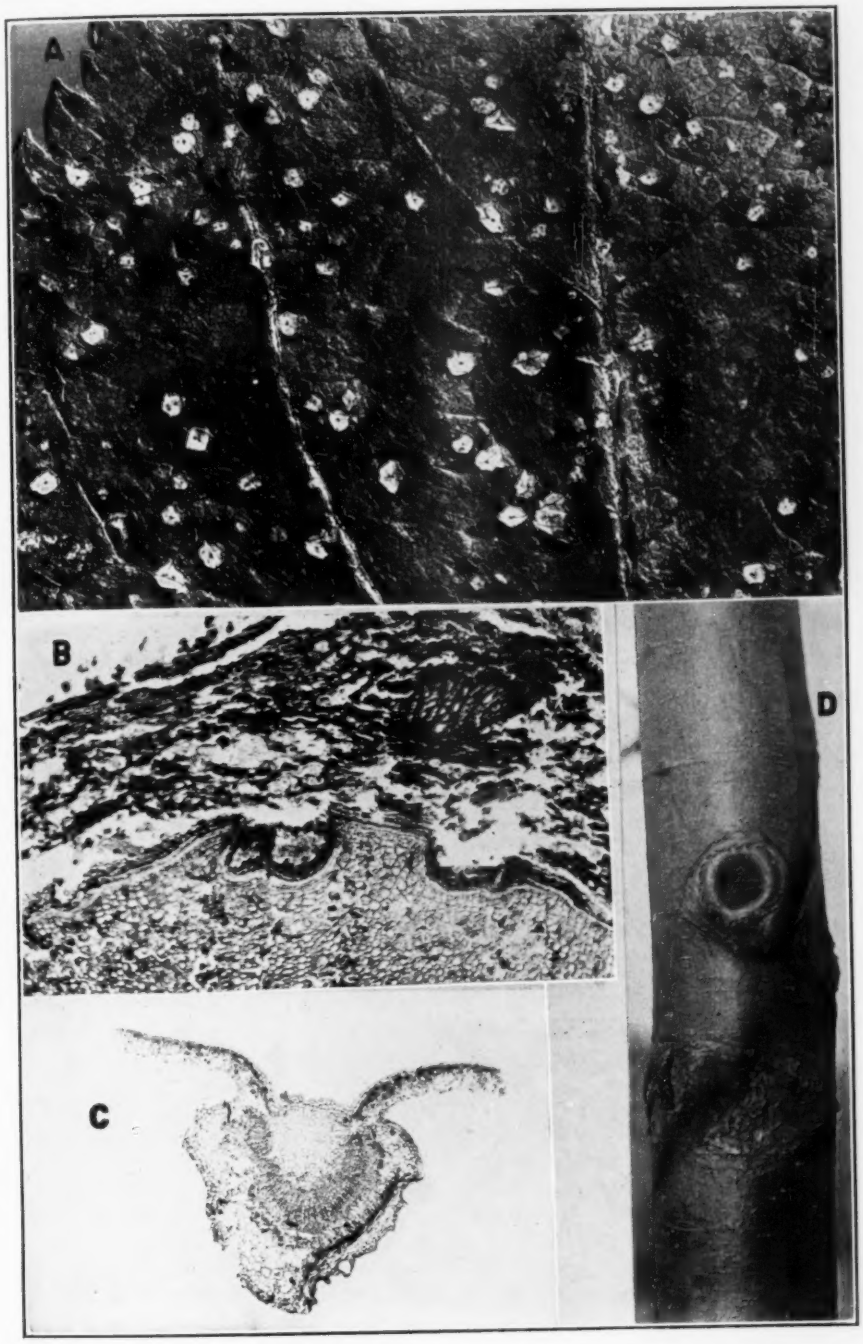
FIG. B. Photomicrograph of a section through a spur canker from a three-year-old twig collected June 2, 1921. The dead cankered area is almost exfoliated and the periderm layer below, formed in the season when infection occurred, has eventually become exposed.

FIG. C. Photomicrograph of a section through an affected mid-vein of an apple leaf showing the sunken irregular surface of the lesion, the abscission layer between healthy and diseased areas, and the compact columnar arrangement of small parenchyma cells beneath.

FIG. D. Apple blotch canker on seven-year-old branch of a Duchess apple tree. Exfoliation of the canker is almost complete yet the fungus still persists in the margin.



PATHOLOGIC HISTOLOGY OF APPLE BLOTCH



PATHOLOGIC HISTOLOGY OF APPLE BLOTCH

CONTROLLING ONION SMUT WITH KALIMAT

P. J. ANDERSON

The use of formaldehyde in controlling onion smut is attended by the danger of killing, or preventing the germination of, the onion seeds. There is a certain amount of loss from this source every season, and under certain moisture conditions of the soil (described in a previous publication[1]) it becomes serious.

Such injury may be largely overcome by changing the rate of dilution and application of the formaldehyde according to the moisture condition of the soil.[2] Control operations would be much simplified, however, if some chemical could be found which could be substituted for formaldehyde and which would give equally good control without the disadvantage of having to change formulas according to the weather or risking a loss of stand. Three years ago the writer began a search for such a substitute and has experimented with a large number of chemicals, some of which have some merit, but, with one exception, none gave a percentage of control equal to the formaldehyde solution. Kalimat,[3] the one exception, however, in experiments during the last two years has given just as good control as formaldehyde used according to the same formula and has caused little or no injury even where formaldehyde caused serious loss.

In the present article only the experiments in which these two chemicals were compared are described. Experiments with other substances are still in progress and will be described in a future publication.

PRELIMINARY GREENHOUSE EXPERIMENTS

When a sample of kalimat was first received, rows of seed planted in two small flats of smut-infested soil in the greenhouse were treated with the chemical diluted according to the formulas of 1–50–3000 (= 1 pint chemical in 50 pints of water on 3000 feet of row), and 1–100–3000. This initial test, applied to each chemical under experimentation, was merely for the

[1] Anderson, P. J. The relation of soil moisture to formaldehyde injury of onion seedlings. Phytopath. 13: 392–403. *2 fig.* 1923.

[2] See Mass. Agr. Col. Ext. Leaflet 79, "How to prevent onion smut." 1924.

[3] Kalimat is a formaldehyde preparation recently patented in Germany and used in that country for control of smuts of cereals. It is now marketed in this country by the Chicago Process Co., 2602–2620 North Western Avenue, Chicago, Illinois. It contains other substances besides formaldehyde and it is claimed by the manufacturers that it not only does not impair germination of the seed but that it actually increases the percentage of germination.

purpose of seeing whether it gave indication of fungicidal value warranting further tests. The seed was not counted but an untreated check row was planted in each flat. Both formulas gave good control since only a few of the plants in the treated rows were smutted while practically all the check plants were diseased.

In the next experiment, additional formulas were tried in comparison with the same formulas of formaldehyde. Eighteen rows, each four feet long and containing two hundred seeds, were planted in a greenhouse bench. The soil had been brought from a badly infested field and had a moisture content of 20 per cent when the seed was planted. It was observed when the seedlings first appeared above ground that both the formaldehyde and the kalimat caused a germination delay of 24–48 hours. This was also observed in later experiments and has been observed in all of our formaldehyde treatments. It is probably of no serious import. A few days later, even before smut appeared, damping-off began in the untreated rows but was almost absent from the rows treated with either chemical. For many years it had been observed in the experiments that this damping-off commonly accompanies or precedes the smut and that both diseases are controlled by the same fungicides. Smutted plants are more susceptible to damping-off but the latter disease seems to be always associated with other fungi such as Pythium, Rhizoctonia and Fusarium. The plants which died from damping-off or smut were removed and counted as they dropped. At the end of eight weeks the value of each treatment was determined by counting the number of healthy plants which were still standing. This seems to be the most accurate method of recording the benefits of any treatment for smut because it expresses most directly the amount of benefit which the grower may expect. Counting the smutted plants in an early stage is not accurate because many smutted seedlings recover and make good onions and also because it is frequently impossible to determine, when a plant dies, whether it was killed by smut or by damping-off. The formulas of treatment and results of this experiment are presented in table 1. The results showed that: (1) There was no injury to the seeds by kalimat in any formula; (2) There was apparently a slight injury from formaldehyde when the concentrated formulas of 1–50–3000 and 1–50–4000 were used. None of the other formulas caused any injury but since this soil contained 20 per cent of moisture when the seed was planted, no considerable injury was anticipated.. (3) Kalimat controlled smut just as well as formaldehyde in all dilutions.

Since it had thus been demonstrated that kalimat controlled smut just as well as formaldehyde in a fairly moist soil, the next step was to see what the effect would be in drier soils. In order to keep the soil moisture con-

stant in these tests, pots of galvanized iron, 12 inches in diameter, were filled with infested soil and then brought up to the desired weight every day by adding water. The soil had a water holding capacity of 64 per cent of

TABLE 1.—*Results from use of kalimat as compared with formaldehyde. Greenhouse test in soil of 20 per cent moisture content*

Chemical	Formula	Total germination	Healthy plants left after 8 weeks
Kalimat	1–50–3000	167	121
Formaldehyde	1–50–3000	150	108
Check		164	12
Kalimat	1–50–4000	165	109
Formaldehyde	1–50–4000	141	79
Check		160	5
Kalimat	1–50–5000	170	103
Formaldehyde	1–50–5000	165	110
Check		165	9
Kalimat	1–100–3000	170	109
Formaldehyde	1–100–3000	172	119
Check		166	3
Kalimat	1–100–4000	164	114
Formaldehyde	1–100–4000	170	96
Check		161	3
Kalimat	1–100–5000	156	83
Formaldehyde	1–100–5000	161	65
Check		164	0

the dry weight. One hundred seeds were planted in each of 15 pots. Three pots were kept at 10 per cent moisture, three at 12, 14, 16 and 18 per cent, respectively. One of each group of three was treated with formaldehyde of the formula 1–50–3000, one with kalimat of the same formula and the third was left without treatment. Only a few seedlings came up in the pots of 10 per cent moisture. It was therefore apparent that this soil was too dry for good germination and these pots were discarded. Smutted and damped-off plants were counted and removed as they died just as in the preceding experiment and at the end of eight weeks the healthy plants still standing were counted. The results are presented in table 2.

Both kalimat and formaldhyde gave perfect control in this test since no plant in any treated pot died from smut. On the other hand, the percentage of infection in the untreated pots was almost total. The differences

in the number of plants at the end of eight weeks are, therefore, an expression of the amount of chemical injury and are not affected by any smut loss. The injury from formaldehyde increased with the dryness of the soil as was anticipated in view of our previous results (Phytopath. **13** : 402). Kalimat, on the other hand, caused no decrease in germination, the percentage of seedlings being as high or higher than for the untreated check (except for the 18 per cent moisture where there was an abnormally high germination in the check).

TABLE 2.—*Results of treating the seeds with formaldehyde and kalimat in soils of different moisture content*

Moisture Percentage	Chemical	Number of seedlings which came up	Healthy plants at end of 8 weeks
12%	Kalimat	45	45
	Formaldehyde	24	24
	Check	25	3
14%	Kalimat	54	54
	Formaldehyde	27	27
	Check	56	3
16%	Kalimat	74	74
	Formaldehyde	42	39
	Check	50	1
18%	Kalimat	68	66
	Formaldehyde	65	65
	Check	91	0

These results indicate that in a dry soil the chemical injury is largely eliminated when kalimat is substituted for formaldehyde.

FIELD EXPERIMENTS

Kalimat was used in a small way in our field tests of 1923 on four rows and more extensively in 1924. In 1923 the soil was very dry and the weather remained dry and windy for three days after planting. These conditions caused the most serious injury from formaldehyde which has occurred in any of the experiments during six years. Every formula of application caused some injury while the most concentrated formula, 1–50–3000, ruined the crop where it was applied. At the end of the season these latter rows did not have even as many sound onions to harvest as the untreated rows. Two rows, each seventy feet long, were treated with kalimat

1–50–3000 and two similar rows with kalimat 1–100–3000. When the seedlings came up there was evidence of some chemical injury from the 1–50–3000 formula but none from the more dilute formula. Just before harvesting, the only rows in the whole field which seemed to have a normal number of onions were those treated with kalimat. The results are presented in table 3.

TABLE 3.—*Field experiment of 1923*

Chemical	Formula	Number of rows	Average number good onions harvested per row
Kalimat	1–50–3000	2	180
Formaldehyde	1–50–3000	4	70
Kalimat	1–100–3000	2	280
Formaldehyde	1–100–3000	4	112
Check		4	102

TABLE 4.—*Comparison of kalimat and formaldehyde in the field tests of 1924. Each row 70 feet long*

Chemical	Formula	Number of rows treated	Average number of seedlings per row which came up	Average sound onions harvested per row
Check		12	539	122
Kalimat	1–50–5000	8	544	340
Formaldehyde	1–50–5000	8	538	309
Kalimat	1–50–4000	8	625	432
Formaldehyde	1–50–4000	8	525	331
Kalimat	1–50–3000	4	516	380
Formaldehyde	1–50–3000	4	469	285
Kalimat	1–100–3000	4	593	331
Formaldehyde	1–100–3000	4	416	293

In 1924, kalimat was tested more extensively and each treatment duplicated on four to eight rows in different parts of the field. Alongside each two rows treated with kalimat were two rows treated with formaldehyde of the same formula. Planting was purposely delayed until a time when the soil was very dry in order to test the kalimat on a dry soil, but rains

began within a few hours after planting, with the result that the injury from formaldehyde was not very serious. Nevertheless, an inspection of table 4 shows that there was considerable loss from the more concentrated formaldehyde formulas while there was no loss in any case with the kalimat except for a possible slight one with the 1–50–3000 formula. If one uses the number of onions harvested at the close of the season as the basis of comparison, the results in every case are strikingly in favor of kalimat. The best results were obtained with the 1–50–4000 formula.

CONCLUSION

In all tests during the last two years, kalimat has controlled onion smut just as well as formaldehyde. It is superior to formaldehyde however not on account of its fungicidal properties but on account of its comparative safety when used in concentrated solutions or when used in very dry soils. Under these same conditions formaldehyde frequently causes serious injury.

The claim of the manufacturers that the percentage of germination is increased by the use of kalimat would seem at first to have some support in the data presented in tables 1, 2 and 4. Of the 14 comparisons between treated and check rows tabulated there, 10 show that there were more seedlings on the treated than on the untreated rows. This does not necessarily mean however that kalimat stimulated germination of the seeds; it may have merely killed off some organisms which naturally destroy the seedlings before they reach the surface of the soil.

The only objection to the use of kalimat is that it is rather expensive at present. It may be obtained for about $1.00 per pint. In larger quantities however it could probably be obtained at a lower price. If any considerable demand for it should be developed, the cost of production will be lowered or other substances embodying the same protective principle will appear on the market.

Of the formulas tried, probably the 1–50–4000 could be recommended as giving excellent control and as being economical of labor and material. This would require about five quarts of kalimat per acre of onions when the rows are thirteen inches apart. In extremely dry soil the same amount applied in greater dilution might have some advantage.

DEPARTMENT OF BOTANY,
MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION

CELLULAR INTERACTION BETWEEN HOST AND PARASITE

CLIFFORD H. FARR

WITH TWELVE FIGURES IN THE TEXT

In the study of the normal development of higher organisms, important consideration is recently being given to the effects of one cell upon another. These effects may be in the nature of stresses and strains brought about by one cell exerting pressure or tension upon a neighboring cell, or they may be chemical effects. In such studies one method of attack is in exposing cells to certain external mechanical or chemical stimuli and observing the alteration made by the cell. Another method is to investigate the effects of one organism upon another in close association with it. A number of investigators have been working recently upon the pressure exerted by a fungus hypha in pushing through the cell wall of its host. It occurred to the writer that further light might be thrown upon this question of cellular interaction, if root-hairs of susceptible and resistant host plants were grown with fungus hyphae under microscopic observation.

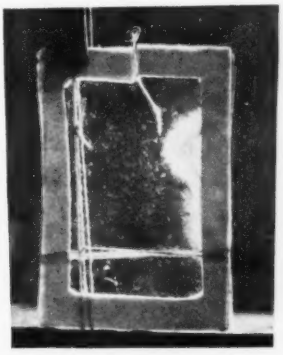


FIG. 1. Mount ready for microscope, containing tomato seedling and wilt fungus.

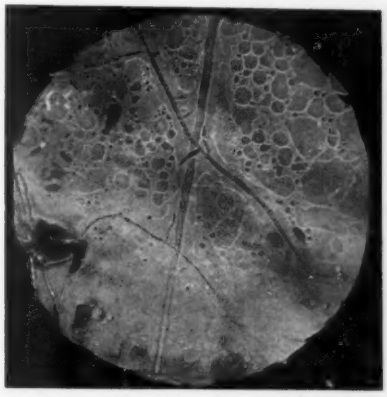


FIG. 2. Hyphae of wilt fungus sending out numerous branches to root-hairs of Stone tomato.

For this purpose cultures of *Fusarium lycopersici* were furnished through the courtesy of Dr. Fred J. Pritchard, of the Bureau of Plant Industry. They were grown on agar in damp chamber mounts, which I[1] have described recently, and into these mounts were inserted seedlings of tomato.

[1] Farr, C. H. A damp chamber for microscopes. Science **56**: 227–228. 1922.

(Fig. 1.) The resistant varieties of tomatoes used were Marvel, Columbia, and Norton, described by Pritchard,[2] and Stone was used as a susceptible variety, which Pritchard reports as becoming 96.1 per cent infected, and which Edgerton and Moreland[3] state is extremely susceptible. While the tomato wilt fungus has not been found to penetrate the root hairs of the tomato, yet Tisdale[4] has shown that *Fusarium lini* does penetrate the root-hairs of both susceptible and resistant varieties of flax, and that the same is true of *Fusarium conglutinans* on cabbage.[5] The difference between re-

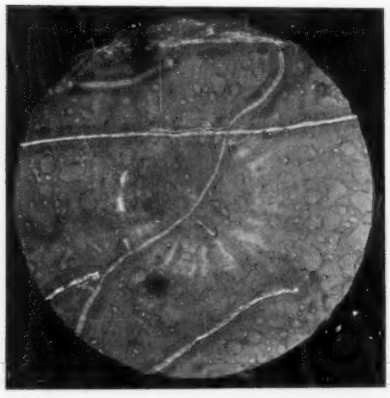


FIG. 3. Branches of hyphae extending along opposite sides of a root-hair.

FIG. 4. Hyphae sending out branches toward root-hair at some distance.

sistant and susceptible varieties in these cases seemed to be that the former develop a corky tissue after infection, walling off the parasite. Although penetration of the tomato wilt fungus apparently does not occur through the root-hairs, and although in this case also both resistant and susceptible varieties are infected, yet it is possible that some difference in reaction between the host and parasite may be discovered. The accompanying photomicrographs show the results of the experiments performed. Figures 2 to 6, inclusive, present root-hairs of the Stone variety of tomatoes being attacked by hyphae of the tomato wilt fungus. Although careful search was made of numerous instances like these in which the hyphae were closely

[2] Pritchard, F. J. Development of wilt-resistant tomatoes. U. S. Dept. of Agriculture Bull. 1015. *18 p., 10 pl.* 1922.

[3] Edgerton, C. W., and Moreland, C. C. Tomato wilt. Louisiana Agric. Exp. Sta. Bull. 174. *54 p., 19 fig.* 1922.

[4] Tisdale, W. H. Flax wilt: A study of the nature and inheritance of wilt resistance. Jour. Agric. Res. **11**: 573–605. 1917.

[5] Jones, L. R., J. C. Walker and W. B. Tisdale. Fusarium resistant cabbage. Wisc. Agric. Exp. Sta. Res. Bull. 48. *34 p., 10 fig.* 1920.

appressed to the root-hair, no instance was seen in which there seemed conclusive evidence that the hyphae had penetrated into a living root-hair. A few instances of hyphae branching in the vicinity of root-hairs might be regarded as coincidence. But the many instances observed, and such instances of obvious reaction on the part of the hyphae as is seen with both hyphae in figure 2, make it seem certain that the branching of the hypha is in direct response to a stimulus received in some way from the root-hair. In some instances, as in figures 2, 3 and 4, the hyphae and root-hairs are in the film of water on the coverglass of the mount. In such instances the stimulus may be transported in the aquaeous solution. In other cases,

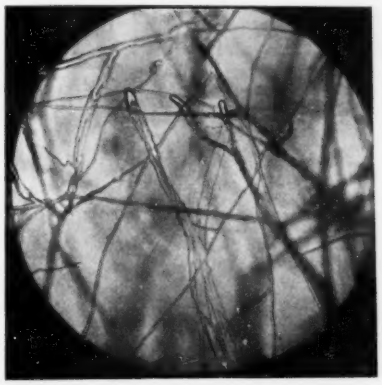


FIG. 5. Near center may be seen the close association of hyphae about root-hair.

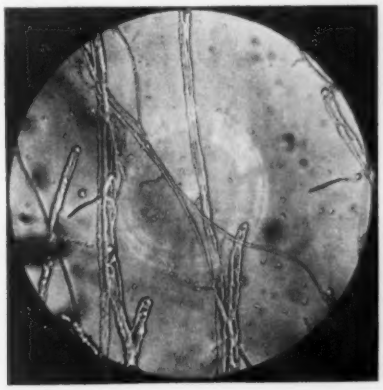


FIG. 6. Hyphae branching toward hairs.

however, as in figures 5 and 6, both hyphae and root-hairs are in mid air in the damp chamber. The stimulus must in these cases operate through the atmosphere. The response of the fungus is in the nature of a morphotic movement. No tropic or nastic movements of the hyphae were detected. The first evidence of a response is the sending out of branches in the direction of the root-hair.

Cultures were made of the Marvel, Columbia, and Norton varieties of tomatoes resistant to wilt. In no instance were the morphotic reactions described above seen in these. Figure 7 shows two hyphae crossing two root-hairs, without giving any response. Figures 8, 9, and 10 are of the same hypha and root-hair taken at fifteen minute intervals, showing the rate at which the hypha and root-hair elongate. It is evident from these pictures that the hypha grows several times faster than the root-hair.

An occasional root was found which showed a marked response to the presence of the fungus. In all cases these were roots of Stone seedlings.

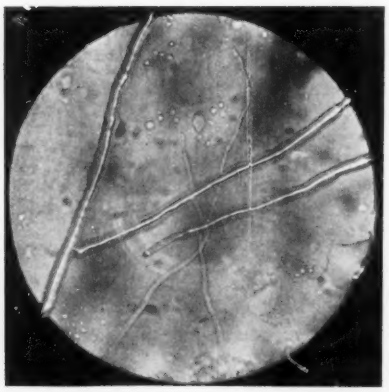


FIG. 7. Hyphae of tomato wilt fungus crossing root-hairs of Marvel.

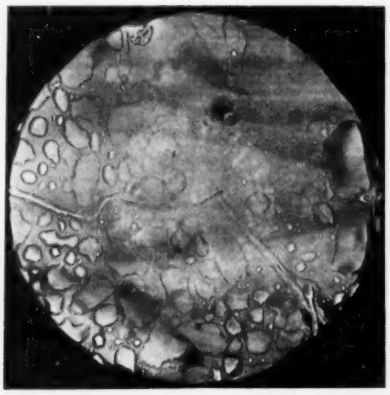


FIG. 8. Hypha of *Fusarium lycopersici* just crossing root-hair of Marvel at 11:45 p. m.

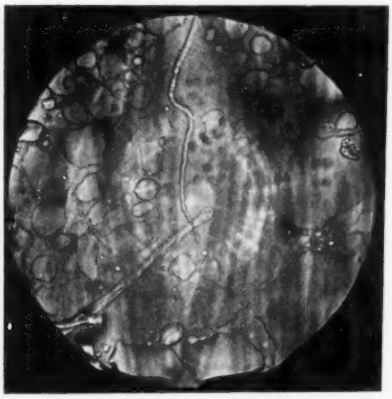


FIG. 9. Another view of same hypha and root-hair as in fig. 8, at midnight.

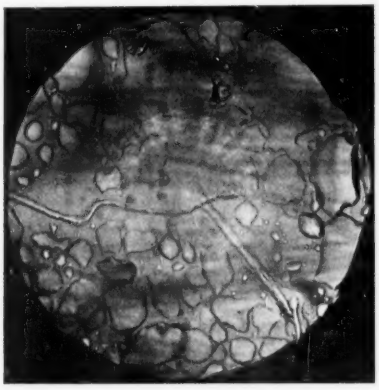


FIG. 10. Another view of same hypha and root-hair as in fig. 9, at 12:15 a. m.

Figure 11 shows such a root, with no root-hairs on the lower portion of the region of absorption on the side from which the root-hairs are approaching, while normal root-hairs have developed on the other side. At the time this formation had begun long before the hypha came in contact with the root but in a different plane of focus, and hence do not show in this picture. It is evident, however, that the reaction of the root in inhibiting root-hair formation had begun long before the hyphae came in contact with the root or any of the root-hairs. It would seem then that the stimulus is trans-

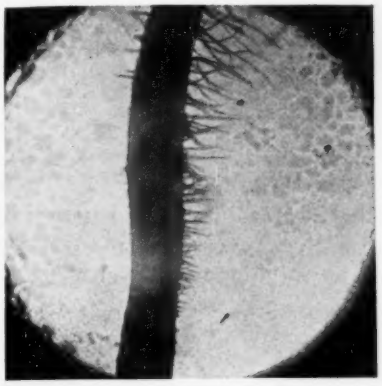


FIG. 11. Root of Stone being approached by hyphae from the left. Note absence of root-hairs on that side.

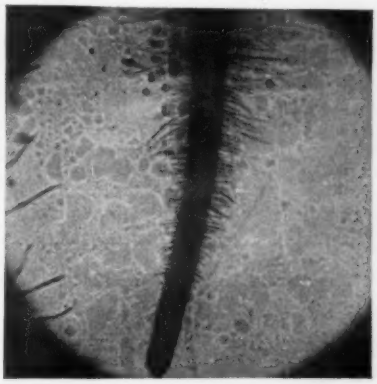


FIG. 12. A root of Marvel, showing normal development of root-hairs even in the presence of the wilt fungus.

ferred from the fungus to the root through the atmosphere of the damp chamber. Miss Snow[6] and others have shown the inhibiting effect of lack of oxygen upon the production of root-hairs. This is a possible explanation here, though it is difficult to understand how the concentration could be enough different on the two sides of the root to account for the result. Roots of Marvel did not show this response to the fungus, as is seen in figure 12. It is thus shown that the susceptible varieties of host show a morphotic reaction to the parasite, while the parasite also shows a morphotic reaction toward the susceptible host.

[6] Snow, L. M. The development of root hairs. Bot. Gaz. **40**: 12–48. *pl. 1, fig. 10–15.* 1905.

MEASURING WATER FLOW INTERFERENCE IN CERTAIN GALL AND VASCULAR DISEASES

I. E. Melhus, J. H. Muncie and Wm. T. H. Ho

With One Figure in the Text

The nature and extent of the injury caused by gall and wilt diseases are not well defined. The symptoms associated with these types of diseases suggest as one of the causes, some disturbance of, or interference with the sap flow in the vascular system. This relationship of galls to sap flow interference was first studied in crown gall (*Bacterium tumefaciens*) on young apple trees and tomato plants. Again this phenomenon of water flow and wilting incident to the wilt diseases was studied in cabbage yellows (*Fusarium conglutinans*), and an alfalfa wilt (causal agent unknown). The flow interference in plants affected with the diseases enumerated, was measured, using a special piece of apparatus designated a fluometer. In addition to detecting the presence or absence of water flow interference, this apparatus reveals the degree of functioning of the ducts and their actual distribution when water soluble stains are employed. A description of the apparatus and some of the data obtained are presented.

The apparatus, as illustrated in the accompanying figure, consists essentially of a filter pump (7) which exhausts the air from the system. A filtering flask (4) is used as a trap to prevent water from backing into the tubes when the pump is shut off without releasing the suction. A mercury column (1), the base of which extends into a volume of mercury in a filtering flask is coupled to the system. By controlling the height of this column, a constant pressure can be maintained. A side delivery burette (8) gauges the amount of water flow through the stem and a separatory funnel (5) serves as a reservoir for water that can be admitted into the burette (8), after the stem is attached and before suction is applied.

The manipulation is very simple. Clamp number 2 is closed and the pump is started; this lowers the mercury column to the desired pressure in about two minutes with average water pressure on the pump. A section of plant stem (6) of standardized length, is then attached to the end of the burette (8) by a short piece of rubber tubing and immersed in water in the jar (9). Water is then admitted from the reservoir (5) to bring the level up to any desired graduation on the burette. Clamp (10) is tightly closed, then clamp (3) is closed and finally clamp (2) is opened. Suction then begins on the stem. When clamp (2) is opened the mercury momentarily rises but returns to its former lower level when equilibrium is established.

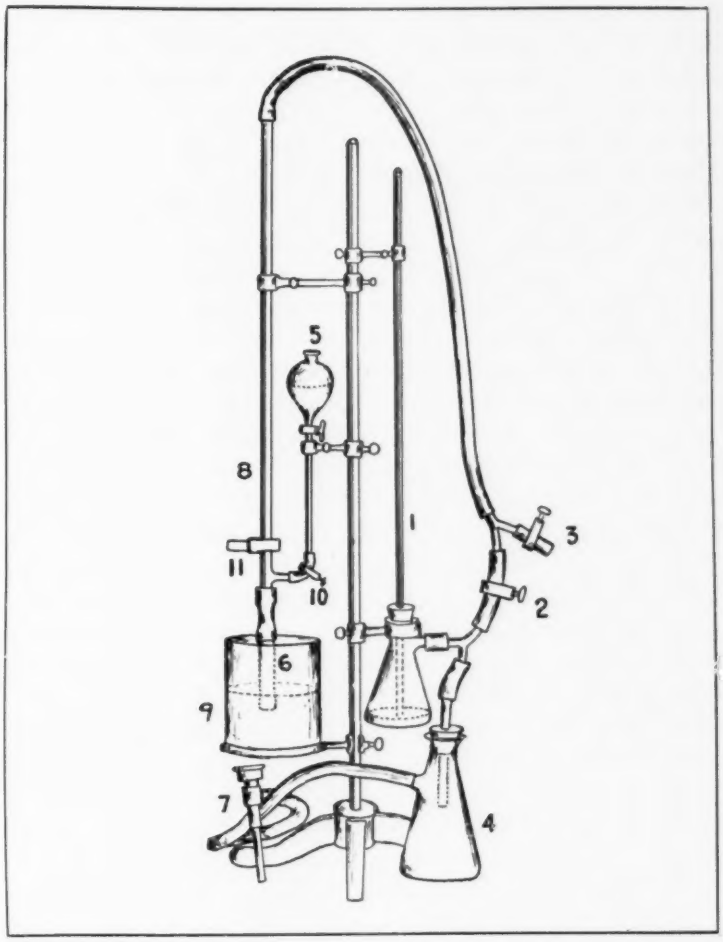


FIG. 1. Fluometer.—1. Mercury column showing reduced pressure obtained by the filter pump (7).—2. Clamp for maintaining vacuum in the mercury flask. By closing this clamp the suction on the system is held while a new specimen is inserted in the rubber connection on the end of the burette (8).—3. Clamp for releasing the vacuum on the burette while changing specimens.—4. Filter flask which serves to prevent back-water from entering the system after the filter pump is shut off.—5. Separatory funnel reservoir. Water is admitted from this funnel into the burette (8) to any desired graduation before suction is applied.—6. The specimen attached by a rubber tubing connection to the burette (8).—7. Filter pump by means of which vacuum is produced in the system.—8. Graduated burette into which the water is pulled through the specimen.—9. Jar of water into which the end of the specimen is placed.—10. Clamp for closing the connection between the reservoir (5) and the burette (8).—11. Meniscus reader marking the water level in the burette (8) before suction is applied. This facilitates reading the water flow through the specimen.

After suction begins, bubbles frequently rise from the stem into the burette; these, however, are soon exhausted and the water in the burette slowly rises as its volume is increased by intake through ducts in the stem. The rate of this flow can then be timed. A meniscus reader (11) marking the height of the water column before suction is applied, facilitates recording the volume per minute passing into burette.

After the flow has been observed, clamp (2) is closed in order to maintain suction on the mercury column, and clamp (3) is opened to release the vacuum in the rest of the system. A new piece of stem can then be attached to the burette end and the process repeated. By keeping clamp (2) closed when attaching a new stem to the burette, the vacuum in the mercury flask is maintained thus saving several minutes on each trial that would otherwise be spent in lowering the column.

By the use of the above described apparatus, the rate of water flow in 100 galled and 100 healthy apple trees was determined. The trees used in these tests were two year "cut backs." A section of each tree was selected 15 cm. long including the union and a part of the scion and stock. All lateral roots were cut off close to the stock. The reading on the burette at the end of the second five minute period was taken as the rate of flow.

In the following table are given the mean rates of flow through the sections of galled and healthy apples. It will be noted that there is a difference in the rate of flow through galled and healthy trees. The average reduction in the rate of flow in the trees cited in the above table is 30 per cent. It should be noted that there is considerable variation in rate of flow in the galled trees. This is probably an indication of the extent of interference by the gall in the infected tree.

TABLE 1.—*Mean rate of flow in galled and healthy apple trees*

Diameter of piece	Galled trees Rate of flow in 5 minutes	Healthy trees Rate of flow in 5 minutes
1.6 cm.	2.7 cc.	4.3 cc.
1.7	5.1	6.9
1.8	4.6	7.8
1.9	7.4	8.2
2.0	5.0	9.2
Weighted mean	5.3 cc.	7.4 cc.

This apparatus is readily adapted to the detection of interference or stoppage of the water flow in herbaceous as well as woody plants. This is shown in the results on cabbage plants affected with cabbage yellows, (*Fusarium conglutinans*). The symptoms associated with this disease

seem to be due to the partial or complete stoppage of the vascular system. Those plants that show only a partial stoppage, die slowly, while those showing no flow succumb quickly. In the following table, representative data are presented.

TABLE 2.—*Rate of flow through healthy and yellows affected cabbage stems*

Diseased		Healthy	
Diameter of stems	Rate of flow in five minutes	Diameter of stems	Rate of flow in five minutes
14 mm.	1.7 cc.	14 mm.	17.2 cc.
15	0.5	15	10.0
15	2.0	15	11.4
15	2.0	15	15.0
15	2.3	16	7.2
16	0.6	16	17.2
16	1.0	16	23.0
16	1.0	16	24.5
16	4.5	16	25.0
17	3.0	17	9.0
Average 15.5 mm.	1.86 cc.	15.6 mm.	16.02 cc.

From the above table we observe that the mean flow of 10 healthy cabbage stems, with an average diameter of 15.6 mm. is 16.02 c.c. in 5 minutes, while that of 10 diseased ones, with an average diameter of 15.5 mm. is only 1.86 c.c. for the same length of time.

Not only is this apparatus adapted to studying the rate of flow in stems, but also in fleshy roots. The data obtained in the study of an alfalfa wilt disease now under investigation in our laboratory show a typical response. The vessels of the wilt infected plants are filled with a gummy exudate and micro-organisms which apparently cause a complete plugging of most of the ducts. The comparative response of healthy and wilt affected plants is shown below:

TABLE 3.—*Mean rate of flow in diseased and healthy alfalfa roots*

	Diseased roots	Healthy roots
Circumference of root	Rate of flow per minute	Rate of flow per minute
1.0 cm.	0.85 c.c.	2.75 c.c.
1.1	0.92	2.92
1.2	0.72	3.90
1.3	3.50	4.50
1.6	3.30	9.00
Weighted mean	0.97	3.85

The data presented suggest that the vascular distortion caused by crown gall (*Bacterium tumefaciens*) in the trees studied, induces sufficient interference to reduce the rate of water flow 30 per cent. The wilting incident to field infection of cabbage with *Fusarium conglutinans* is due to a partial or complete stoppage of the ducts in the vascular system. The alfalfa wilt results from a vascular plugging. In this case, a gummy exudate filling the ducts is readily detected in stained sections.

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A METHOD OF INCREASING THE EFFICIENCY OF FILTER CYLINDERS

H. H. McKINNEY

WITH ONE FIGURE IN THE TEXT

The efficiency of filter cylinders is greatly reduced when any part of the filter is not covered by the fluid being filtered. This is due to the reduction in the amount of filter surface and to the decreased efficiency of the aspirator on account of air leakage through the exposed portion of the filter. The use of mantles of small diameter aids in the reduction of this inefficiency, but this practice makes it necessary for the operator to remain with the apparatus constantly to keep the mantle filled.

In order to eliminate these difficulties, the writer has devised a glass tube, for convenience called "filter tube," which has an inside diameter slightly larger than the outside diameter of the filter to be used. The bottom of this filter tube is open and the top is designed to be closed, as shown in figure 1. The fluid to be filtered is placed in the mantle and the filter tube is placed over the filter, the upper end being left open to allow the air to escape. In case the mantle is not full, the excess air in the filter tube is drawn off through the opening and the top is then closed. Thus the fluid fills the filter tube and covers the filter as long as the bottom opening of the filter tube is under the fluid.

This method is especially advantageous when it is desired to filter fluids containing much colloidal material, since it enables the efficient use of large filters. In the filtration of plant juice containing chlorophyll, the writer uses large 2″ x 10″, coarse, diatomaceous filters with filter tubes, as shown in figure 1 *a* and *b*. The chlorophyll deposit is removed frequently from the surface of the filter and the resulting clear filtrate is later passed through fine bacteria-proof filters.

When using small-sized filters, the writer employs filter tubes made of short lengths of ordinary large-sized glass tubing, or test tubes which have their bottoms cut off. The upper end of such a tube is closed with a rubber stopper with single opening. In this is placed a ground-glass stop cock, or a short glass tube carrying a piece of rubber tubing with clamp cock. When using the Livingston atmometer cup as a filter, it usually is necessary to pour agar into the mantle in order to seal all possible openings which may occur around its base, and to raise the bottom of the mantle to the unglazed portion of the cup. When using a filter tube with such a setup, it is necessary to use a hot wax, such as paraffin, for sealing

instead of agar, as agar is too soft and is lifted out of place by the force of the fluid entering the base of the filter tube.

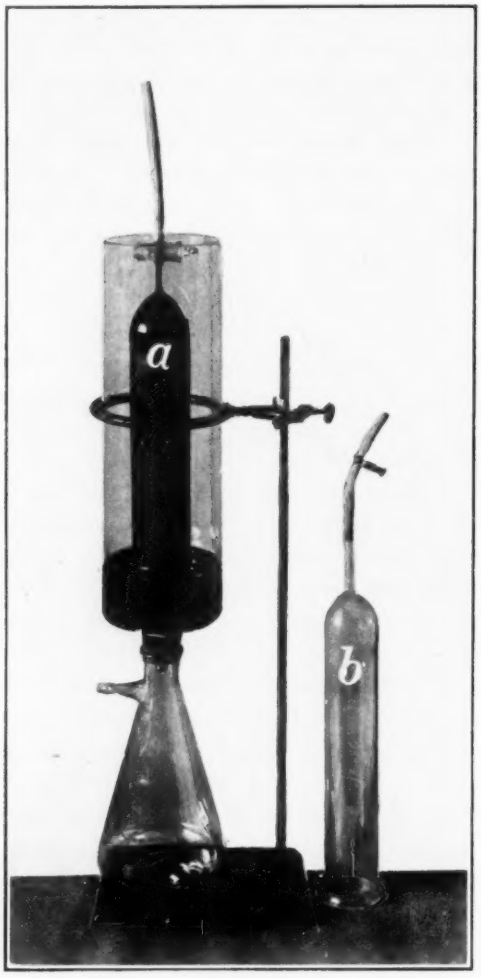


FIG. 1. Large-sized filter with glass-stoppered filter tube in position (*a*). Large-sized filter tube (*b*) equipped with plain glass tubing outlet. A short piece of rubber tubing and clamp cock provide an inexpensive sealing device.

OFFICE OF CEREAL INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
AND UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

PHYTOPATHOLOGICAL NOTES

Java Gum Disease of Sugar Cane Identical to Leaf Scald of Australia. The writers, after a conference in Honolulu in which formalin specimens and photographs have been compared, wish to point out the similarity between the disease of sugar cane known as leaf scald in Australia and the gum disease described by Miss Wilbrink[1] which occurs in both Java and the Philippine Islands. The pallid-white, evenly-edged, leaf streaks, reddened vascular bundles, production of shoots from the nodes and general leaf appearance are apparently identical in both diseases. The work, soon to be published, of the first mentioned of the writers of this note, shows the great similarity between the causal organism of leaf scald in Australia and the organism described as the cause of the Java gum disease by Miss Wilbrink.

The Java gum disease as described by Miss Wilbrink is decidedly different from Cobb's gumming disease of sugar cane and since it may lead to confusion to continue with such a name, it seems desirable to adopt the rather descriptive name leaf scald as used in Australia for this trouble.

Leaf scald of sugar cane has been recorded, to the present time, only in Australia, Fiji, Java and the Philippines. We regard it as one of the most destructive of the diseases of sugar cane, and sugar countries of the western hemisphere should exert every possible effort to prevent its introduction.—D. S. North, H. Atherton Lee.

Personals: There died suddenly, on August 20, on the occasion of an excursion of the Hamburg Horticultural Society, Prof. Dr. Carl Brick, the Director of the Station für Pflanzenschutz, Hamburg, Germany. Brick was born in 1863 at Stolp, in Pomerania, and studied Natural Sciences at the University of Breslau. In 1888 he secured his Ph.D. on "Contributions to the biology and comparative anatomy of Baltic shore plants." He then went to the Botanical Museum, Hamburg, and later became first assistant at Karlsruhe, where he lectured on Forest Botany and Soil Science. He returned in 1891 to the Hamburg Museum, and in 1898 became director of the new Pflanzenschutzstation, where he successfully worked until his end. Among his many contributions to science there may be mentioned his studies of the parasitic fungi and insects of imported fruit, the fungus flora of the Sachsenwald and diseases of tropical plants.—H. T. G.

[1] Wilbrink, G. De gomziekte van het suikerriet, hare oorzaak en hare bestrijding. Mededeelingen van het Proefstation voor de Java-Suikerindustrie. XXVIII, 2 deel, 1921, p. 1399.

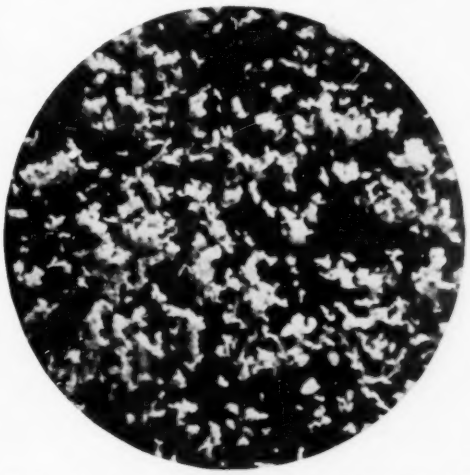
An unusual infection of Polyporus Schweinitzii Fr.—This fungus on living trees is a typical root and butt rot, usually confined to the heartwood of the first ten feet of the trunk although it occasionally extends considerably higher. In all such cases observed, however, the rot column has been continuous from the butt of the tree upward and the infection court always seemed to be in the roots or in old scars just above the ground level.

It was with considerable surprise that at Alder, Pierce Co., Washington, on July 7, 1922, a living Douglas fir (*Pseudotsuga taxfiolia* (Lam.) Br.) with a diameter at breast height, outside the bark, of 60 inches was found with a cluster of *Polyporus schweinitzii* sporophores issuing from an old knot at 51 feet above ground level. The tree had been felled on a logging operation, leaving a stump 4 feet high, and then two logs had been cut out, each 32 feet long. The sporophores occurred 15 feet from the base of the second log but there was no decay in either the base or top of the log. Likewise the stump and first log were free from decay. Obviously the decay was the result of a local infection, probably through the old knot from which the sporophores issued.—J. S. BOYCE.

The November number of Phytopathology was issued December 2, 1924.

Ortho Dusting Sulphur

Photomicrograph showing extreme fineness of Ortho Sulphur
Note that particles do not bunch together



Photomicrograph of best sublimed sulphur showing comparative coarseness compared with Ortho and tendency to bunch together

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